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Exploring the modulatory role of A_{2A} receptors in oligodendrogenesis derived from neural stem/progenitor cells of the subventricular zone.

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Don't dream it, be it.

The Rocky Horror Picture Show

Resumo

Os oligodendrócitos são as células responsáveis pela formação de mielina no sistema nervoso central. A sua membrana rica em colesterol é capaz de revestir axónios, formando a bainha de mielina. A bainha de mielina é uma estrutura espiral multilamelar que protege e fornece isolamento elétrico aos neurónios, permitindo uma maior velocidade de transmissão de potenciais de ação e contribuindo para a sobrevivência neuronal. Apesar da sua importância, os oligodendrócitos são facilmente afetados por stress oxidativo, o que pode levar à desmielinização.

Durante o desenvolvimento e no cérebro adulto, os oligodendrócitos diferenciam-se a partir de células precursoras de oligodendrócitos. Estas células são um subtipo de célula glial, caracterizadas por expressar PDGFRα (*Platelet-Derived Growth Factor α*) e o proteoglicano NG2 (*Neuron-Glial antigen 2*). No cérebro adulto, estas células correspondem a 3 – 8 % do número total de células e são especialmente prevalentes no hipocampo e córtex. Em casos patológicos, as células precursoras de oligodendrócitos podem ser originadas a partir de células estaminais neurais. E é de salientar que a zona subventricular, um nicho rico em células estaminais neurais, é então também uma fonte de células precursoras de oligodendrócitos. Estas células precursoras são capazes de migrar até ao córtex, *corpus callosum*, *estriado* e *fimbria-fornix*.

Células estaminais neurais são células multipotentes, capazes de autorrenovação, e que geram a maioria das células do sistema nervoso – neurónios, astrócitos, e oligodendrócitos. Estas células estaminais neurais podem dividir-se de três maneiras diferentes: simetricamente, originando duas novas células estaminais neurais, o que leva à expansão da população de células estaminais neurais e à sua autorrenovação;

assimetricamente, originando uma célula estaminal neural e uma célula diferenciada, o que contribui para a manutenção da população de células estaminais neurais enquanto ao mesmo tempo permite a diferenciação de novas células; ou novamente simetricamente, originando duas células diferenciadas, o que leva a uma redução da população de células neurais estaminais e à produção aumentada de novas células neurais. A natureza destas novas células vai depender da ativação de vias de sinalização específicas e da presença de moléculas que induzam a diferenciação, podendo estas células ser neurónios (neurogénese) ou células gliais (gliogénese), sendo particularmente importante a formação de novos oligodendrócitos (oligodendrogénese).

No cérebro adulto, as células estaminais neurais existem em zonas específicas, onde a neurogénese e oligodendrogénese são altamente reguladas. Estas zonas cerebrais onde estes processos ocorrem, ou seja, onde se podem encontrar as populações de células estaminais neurais, são os chamados nichos neurogénicos. Existem fundamentalmente dois nichos neurogénicos no cérebro adulto: a zona subventricular dos ventrículos laterais, e a zona subgranular do giro dentado do hipocampo. Especificamente, o nicho neurogénico da zona subventricular é capaz de produzir interneurónios olfativos a partir de neuroblastos que migram pela via rostro-migratória e se diferenciam em interneurónios GABAérgicos e dopaminérgicos no bolbo olfativo. Este nicho é também capaz de originar oligodendrócitos na presença de um insulto desmielinizante, como acontece na esclerose múltipla.

A esclerose múltipla é uma doença inflamatória desmielinizante na qual há morte celular de oligodendrócitos e, consequentemente, as bainhas de mielina no cérebro e na medula espinal estão danificadas, o que leva a falhas na comunicação neuronal. Não existe cure para a esclerose múltipla e a esperança de vida para indivíduos

afetados é cinco a dez anos mais baixa quando comparada com a esperança de vida de indivíduos não afetados.

Quando há um insulto desmielinizante (como na esclerose múltipla), existe morte celular, especialmente de oligodendrócitos. Como dito anteriormente, isto leva a que os neurónios fiquem desprotegidos e incapazes de comunicar normalmente. Quando isto acontece, células precursoras de oligodendrócitos originadas da zona subventricular e presentes no parênquima vão migrar para a zona afetada, onde se vão diferenciar, originando novos oligodendrócitos. No entanto, muitas vezes a taxa de produção de novos oligodendrócitos não é eficiente para colmatar o dano causado pelo dano desmielinizante. Com efeito, em casos como esclerose múltipla, parece haver um bloqueio da remielinização. Assim sendo, torna-se extremamente importante encontrar moduladores de oligodendrogénese que possam funcionar como novos alvos terapêuticos para tratar patologias desmielinizantes.

É neste contexto que decidimos estudar a adenosina e os seus recetores. A adenosina é um nucleósido ubíquo, que desempenha funções importantes na transferência de energia, sob a forma de ATP e ADP; na transdução de sinais, como AMP cíclico; e em neurotransmissão, como neuromodulador. A adenosina tem também funções importantes na neuroproteção, e na regulação de sono e vigília, pressão sanguínea, humor, entre outros. Existem quatro recetores de adenosina: A_1, A_{2A}, A_{2B}, e A_3. Todos estes recetores estão acoplados a proteínas do tipo G que afetam a atividade do adenilato ciclase. Os recetores A_1 e A_3 estão acoplados à proteína $G_{i/o}$, que inibe a atividade do adenilato ciclase, inibindo a produção de AMP cíclico intracelular. Por outro lado, os recetores A_{2A} e A_{2B} estão acoplados à proteína G_s, que estimula o adenilato ciclase, levando a uma maior produção de AMP cíclico intracelular.

Dados preliminares do nosso grupo mostram que a ativação do recetor A_{2A} da adenosina estimula a neurogénese em culturas de células originadas da zona subgranular do giro dentado do hipocampo, mas não tem o mesmo efeito em culturas de células originadas da zona subventricular. Por outro lado, foi encontrada uma maior densidade e proliferação celular na zona subventricular em amostras *post-mortem* de pacientes com esclerose múltipla.

Assim, os objetivos deste trabalho foram traçar a expressão de células de linhagem oligodendrocítica ao longo dos dias em cultura e estudar o efeito da ativação ou bloqueio dos recetores A_{2A} na oligodendrogénese originada da zona subventricular de rato.

Para isso, utilizaram-se ratos Sprague-Dawley com 1-3 dias pós-natal, dissecou-se a zona subventricular e dissociou-se o tecido. As células foram então deixadas a crescer em condições proliferativas, na presença do fator de crescimento EGF (*Epidermal Growth Factor*), durante seis dias. Ao fim destes seis dias, as células deram origem a neuroesferas que foram então plaqueadas em lamelas revestidas com poli-D-lisina e colocadas em condições diferenciativas (sem EGF). Nestas condições, é originada uma cultura mista, com células estaminais neurais, células precursoras, neurónios, astrócitos, e oligodendrócitos. As células foram então incubadas com agonista e/ou antagonista dos recetores A_{2A} durante dois, quatro, ou sete dias. Após estes períodos, foram realizados ensaios de imunocitoquímica e um ensaio enzimático.

Em primeiro lugar, os resultados obtidos mostram que, ao longo dos dias em cultura, as células da linhagem oligodendrocítica se vão diferenciando, originando um número cada vez maior de células precursoras de oligodendrócitos e de oligodendrócitos.

É de realçar que os resultados mostram que a ativação do recetor A_{2A} promove a oligodendrogénese originada da zona subventricular. Especificamente, a ativação do recetor A_{2A} aumenta o número de células positivas para Olig2 (fator de transcrição expresso em todas as células da linhagem oligodendrocítica) a dois e quatro dias *in vitro*; assim como o número de células positivas para NG2 (marcador expresso em células precursoras de oligodendrócitos e em pré-oligodendrócitos) e o número de células positivas para GalC (galactocerebrósido, marcador expresso em oligodendrócitos imaturos e maduros) a dois e sete dias *in vitro*. Para além disto, observou-se que a ativação do recetor A_{2A} leva a um aumento da atividade do enzima CNPase (*2',3'-Cyclic Nucleotide 3'-Phosphodiesterase*), um enzima ligado à mielina e ao processo de mielinização, o que sugere que os novos oligodendrócitos formados são capazes de formar mielina, estando assim funcionalmente intactos.

Deste modo é possível concluir que a ativação do recetor A_{2A} tem um papel modulatório positivo na oligodendrogénese originada da zona subventricular, o que mostra que os recetores A_{2A} podem ser alvos terapêuticos para doenças desmielinizantes.

Palavras-chave: Oligodendrogénese, Zona subventricular, Adenosina, Recetor A_{2A}

Abstract

Neural stem cells (NSCs) are self-renewing, multipotent cells that can generate most of the cells of the nervous system. In fact, NSCs exist in discrete regions of the adult mammalian brain entitled neurogenic niches, mainly located in the subventricular zone (SVZ) and in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG). Moreover, the SVZ is a source of oligodendrocyte precursor cells (OPCs) that can migrate and populate the *corpus callosum*, *striatum*, and *fimbria-fornix* in the adult mouse. Indeed, it has been shown an increased activation of the SVZ in *post-mortem* samples of multiple sclerosis (MS) patients. Given that MS is characterized by demyelination, this could imply that the SVZ is a source of OPCs that will migrate and differentiate into oligodendrocytes in the damaged regions, leading to a restored myelination.

Due to the severe effects of MS, it is extremely important to explore possible therapeutic mechanisms. Adenosine is a neuromodulator with important roles in several physiological processes such as sleep and arousal, cognition, memory and learning, neuroprotection, and regulation of blood pressure and heart rate. Preliminary data from our group has shown that although A_{2A} receptor activation stimulates neurogenesis from DG neural stem/progenitor cells cultures, it has no effect on neuronal differentiation from SVZ cell cultures. Therefore, this study evaluated whether A_{2A} receptor activation could modulate oligodendrogenesis derived from SVZ NSCs.

Results shown that A_{2A} receptor activation promoted oligodendrocyte differentiation, as shown by an increase in the number of Olig2 (a transcription factor expressed throughout the oligodendrocytic lineage)-positive cells at 2 and 4 days *in vitro* and in the number of neuron-glial antigen 2 (NG2, expressed by OPCs, adult

OPCs, and pre-oligodendrocytes) and galactocerebroside (GalC, expressed by immature and mature)-positive cells at 2 and 7 days *in vitro*. Furthermore, it was observed that A_{2A} receptor activation leads to an increase in 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase, a myelin-related enzyme expressed by mature oligodendrocytes) activity, which implies that oligodendrocytes were able to produce myelin.

Taken together, our data suggest that A_{2A} receptor activation has a positive modulatory effect on SVZ-derived oligodendrogenesis, which can be proven useful as a therapeutic approach for demyelinating disorders.

Keywords: Oligodendrogenesis, Subventricular zone, Adenosine, A_{2A} receptor

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Abbreviations

AC	Adenylate cyclase
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
bHLH	Basic helix-loop-helix
BDNF	Brain derived neurotrophic factor
BMP	Bone morphogenic protein
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
cNADP	Cyclic NADP
CNPase	2',3'-cyclic nucleotide 3'-phosphodiesterase
CNS	Central nervous system
DG	Dentate gyrus
DMEM	Dulbecco's modified Eagle's medium
dOL	Dorsally-derived oligodendrocyte
dOPC	Dorsally-derived OPC
EAE	Experimental autoimmune encephalomyelitis
EGF	Epidermal growth factor
ERK	Extracellular signal-regulated kinases
E*x*	Embryonic day *x*
Ezh2	Enhancer of zeste homolog 2
FGF-2	Basic fibroblast growth factor 2
G6P	Glucose-6-phosphate

G6PDH	Glucose-6-phosphate dehydrogenase
GABA	γ -Aminobutyric acid
GalC	Galactocerebroside
G_{olf}	G-olfactory
HBSS	Hank's balanced saline solution
Hdac	Histone deacetylase
ICC	Immunocytochemistry
LTP	Long-term potentiation
MAG	Myelin-associated glycoprotein
MAPK	Mitogen-activated protein kinases
MBP	Myelin basic protein
MES	2-(N-morpholino)ethanesulfonic acid
MS	Multiple sclerosis
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	NADP (reduced form)
NG2	Neuron-glial antigen 2
NSC	Neural stem cell
OB	Olfactory bulb
OPC	Oligodendrocyte precursor cell
PBS	Phosphate-buffered saline
PDGF	Platelet-derived growth factor
PDGFRα	Platelet-derived growth factor receptor α
PI	Propidium iodide
PI3-K	Phosphoinositide 3-kinase
PKA	Protein kinase A

PKC	Protein kinase C
PLP	Myelin proteolipid protein
PNS	Peripheral nervous system
P*x*	Postnatal day *x*
RMS	Rostral migratory stream
SFM	Serum-free medium
SGZ	Subgranular zone of the DG
Shh	Sonic hedgehog
Sox2	Sex determining region Y-box 2
SVZ	Subventricular zone of the lateral ventricles
vOL	Ventrally-derived oligodendrocyte
vOPC	Ventrally-derived OPC

1 Introduction

1.1 CELL TYPES INVOLVED IN OLIGODENDROGENESIS

Oligodendrocytes are the myelin-forming cells of the central nervous system (CNS), while in the peripheral nervous system (PNS) this task is accomplished by Schwann cells. Oligodendrocytes are the last brain cells to be generated during development, making myelination a late event in brain maturation (El Waly *et al*., 2014). Indeed, at the second post-natal day, rodents present mainly pre-oligodendrocytes. The beginning of CNS myelination only occurs at the seventh post-natal day (Barateiro and Fernandes, 2014). By then, the cholesterol-rich membrane produced by oligodendrocytes loops around neuronal axons creating a myelin sheath, which is a multilamellar spiral structure that protects and provides electrical insulation to the neurons, allowing a faster transmission of action potentials along the axons (Bergles *et al*., 2010) and assuring neuronal survival (El Waly *et al*., 2014). While essential for proper brain functioning, oligodendrocytes are easily affected by oxidative stress, and demyelination is often a secondary event to brain lesions or pathologies (El Waly *et al*., 2014).

During CNS development, but also throughout adulthood, oligodendrocytes are generated from oligodendrocyte precursor cells (OPCs). OPCs are a sub-type of glial cell, with a bipolar/tripolar morphology (Coppi *et al*., 2013b), characterized by the expression of the platelet-derived growth factor receptor α (PDGFRα) and the neuron-glial antigen 2 (NG2) proteoglycan (Nishiyama *et al*., 2009). Other known markers for OPCs are the transcription factors Olig1, Olig2, and Nkx2.2 (Fancy *et al*., 2004), as well as A2B5 immunoreactivity (Baracskay *et al*., 2007). However, since these

markers can be expressed in other types of cells, a combination of markers should be used to unambiguously identify OPCs (El Waly *et al*., 2014). In the adult brain, OPCs comprise 3-8% of the total number of cells (Polito and Reynolds, 2005) and are prevalent in the hippocampus and in all layers of the neocortex (Ong and Levine, 1999). In fact, adult OPCs are the largest population of proliferating cells in the CNS (Horner *et al*., 2000).

Oligodendrocyte differentiation from OPCs can be divided in four stages: OPC, pre-oligodendrocyte, immature oligodendrocyte, and mature myelinating oligodendrocyte (Barateiro and Fernandes, 2014; Szuchet *et al*., 2011). The progress through these stages can be tracked by changes in cell morphology and specific cell marker expression. Indeed, the change from OPC to pre-oligodendrocyte is accompanied by the appearance of secondary ramifications in the cell, as well as immunoreactivity to the O4 antigen (Sommer and Schachner, 1981). The next stage, immature oligodendrocyte, can be recognized by a complex multipolar morphology (Back *et al*., 2001) and the expression of oligodendrocytic markers, specifically galactocerebroside (GalC) (Raff *et al*., 1978), and concomitant down regulation of NG2 and PDGFRα expression, while O4 immunoreactivity persists (Yu *et al*., 1994). Finally, fully mature and myelinating oligodendrocytes present a highly ramified morphology and begin expressing myelin-associated proteins, namely 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) (Lee *et al*., 2005), myelin basic protein (MBP), myelin proteolipid protein (PLP), and myelin-associated glycoprotein (MAG) (Baumann and Pham-Dinh, 2001; Coppi *et al*., 2015). Additionally, mature oligodendrocytes progressively lose the capacity to migrate and proliferate, which complements their differentiation process (Barateiro and Fernandes, 2014).

After CNS development new OPCs (also named adult OPCs), presenting the same markers and morphology as the OPCs present during development (Franklin and Ffrench-Constant, 2008), can be produced by adult neural stem cells (NSCs). These NSCs, identifiable by the expression of nestin (Park *et al*., 2010) and the transcription factor sex determining region Y-box 2 (Sox2) (Ellis *et al*., 2004; Thiel, 2013), are self-renewing, multipotent cells that can generate most of the cells of the nervous system, such as neurons, astrocytes, and oligodendrocytes (Alenzi and Bahkali, 2014). NSCs can therefore divide in three different ways: symmetrically, originating two new NSC (expansion, symmetrical self-renewal); asymmetrically, originating one NSC and one differentiated cell (maintenance, asymmetrical self-renewal); or also symmetrically, originating two differentiated cells (extinction, symmetrical commitment) (Casarosa *et al*., 2013). Depending on the activation of specific signalling pathways and the presence of differentiation-inducing molecules, NSCs are capable of differentiating into cells of neuronal (neurogenesis) or glial (gliogenesis) lineages, particularly oligodendrocytes (oligodendrogenesis) (Song *et al*., 2002).

1.2 OLIGODENDROGENESIS DURING DEVELOPMENT AND INTO THE ADULT BRAIN

In the developing forebrain of mice, the entire oligodendrocyte population is generated from three moments of OPC proliferation and migration. The first moment occurs at embryonic day 12.5 (E12.5) and consists of a "wave" of OPC production, originated from ventral ganglionic eminences (Spassky *et al*., 2001; Tekki-Kessaris *et al*., 2001). At E15.5, the second moment takes place, emerging from the lateral and caudal ganglionic eminences (Kessaris *et al*., 2006). Finally, the third moment happens after birth, with origin in the cortex (Kessaris *et al*., 2006). These three

moments are responsible for the generation of most adult oligodendrocytes in mice, which will migrate and populate most of the future brain (El Waly *et al*., 2014).

Given the nature of oligodendrocyte production, one question arises: Are the OPCs involved in these different moments functionally equivalent?

There is evidence that each moment of OPC production can lead to the myelination of distinct brain regions (Tripathi *et al*., 2011), suggesting the existence of functionally different subpopulations of OPCs that serve separate functions. In fact, a study conducted in mice targeted differentially ventrally-derived OPCs (vOPCs) and dorsally-derived OPCs (dOPCs), as well as the oligodendrocytes generated by each class of OPCs (vOLs and dOLs respectively). This study shows that while vOPCs and dOPCs appear to have the same electrical properties, their migration and settling patterns are significantly different, to the point that vOLs and dOLs populate different forebrain and spinal cord regions at different time points during development (for example, while in adulthood the corticospinal and rubrospinal tracts are myelinated by dOLs, during early post-natal life these regions are actually myelinated by vOLs) (Tripathi *et al*., 2011). On the other hand, it has been shown that, if one of these subpopulations is eliminated, neighbouring OPCs of different origins rapidly migrate and proliferate to generate the regular number of oligodendrocytes in the mature brain (Kessaris *et al*., 2006), which could imply that the subpopulations of OPCs are functionally equivalent.

In human CNS development, oligodendrocyte differentiation and maturation follow similar paths to rodents (Ortega *et al*., 2013). This process has its beginning in the second trimester of gestation and spans into birth and adulthood (Jakovcevski *et al*., 2009; Jakovcevski and Zecevic, 2005). Specifically, at 9 gestation weeks, early OPCs (NG2 and PDGFRα positive) arise from the ganglionic eminence, migrating to

the cortex in the following weeks. Late OPCs (or pre-oligodendrocytes), which show O4 immunoreactivity, are first detected in small percentage at 15 gestation weeks, gaining more density in midgestation (c. 20-22 gestation weeks), especially in the subplate layer directly under the cortical plate. Finally, MBP-positive oligodendrocytes are rare at midgestation, but show a steady population growth from that point on. Indeed, the first myelin sheaths can be found around 18 gestation weeks in the thalamus, spreading to the internal capsule at 21 gestation weeks (Jakovcevski *et al*., 2009).

After CNS development, a small fraction of OPCs remains undifferentiated, in an immature slowly proliferative or quiescent state (Dawson *et al*., 2003). These adult OPCs are morphologically equivalent and express the same markers as the OPCs present during development (Franklin and Ffrench-Constant, 2008). However, they differ from the developing OPCs in growth factor responsiveness, migration capacity, and cell cycle length (Psachoulia *et al*., 2009; Wolswijk and Noble, 1989; Wren *et al*., 1992). Their cell density, although stable throughout adult life, is higher in white matter than in grey matter (Dimou *et al*., 2008; Rivers *et al*., 2008). Indeed, it has been shown that adult OPCs present a higher proliferation rate in white matter, which is a possible explanation for the difference in cell density (Dimou *et al*., 2008). It is possible to further divide white matter OPCs and grey matter OPCs by their characteristics. While white matter OPCs are proliferative and eventually lead to adult oligodendrogenesis, grey matter OPCs remain quiescent and immature (Dimou *et al*., 2008).

Given these findings, could adult OPCs be a heterogeneous population, possibly with several distinct functions?

Some studies show that the different characteristics observed in adult OPCs can be the result of environmental signals. Specifically, grey matter environment is described as an inhibitor of OPC proliferation and differentiation, while white matter environment seems to favour OPC maturation (Dawson *et al*., 2003; Dimou *et al*., 2008; Rivers *et al*., 2008). These differences may be linked to intrinsic cell mechanisms or to environmental cues. While there seems to be differences in the local microenvironment surrounding OPCs in white and grey matter, the different characteristics can also be explained by intrinsic mechanisms, such as receptor desensitization. For instance, it is known that, in the developing spinal cord, PDGF-A mRNA has higher expression in grey matter (Calver *et al*., 1998), which can lead to desensitization of the receptor (PDGFRα) and prolonged impairment of grey matter OPCs maturation (Hill *et al*., 2013). However, there are indeed molecular differences between white matter OPCs and grey matter OPCs, namely in the resting membrane potential and ion channels expression (Káradóttir *et al*., 2008; Yuan *et al*., 2002). Concerning the ion channels, two subpopulations of adult OPCs have been described: one completely devoid of voltage-gated Na^+ channels, and another with functional channels, able to react to action potentials. Consequently, this second subtype has the ability to sense neuronal activity through axonal input and is more sensitive to ischemia (Káradóttir *et al*., 2008). Another study corroborating the existence of functionally different subtypes shows that white matter OPCs can originate myelinating oligodendrocytes even if they are transplanted into other brain regions. Gary matter OPCs, in contrast, remain less efficient even if transplanted into white matter (Viganò *et al*., 2013).

1.3 OLIGODENDROGENESIS DERIVED FROM NSCS

Another source of OPCs throughout life, as mentioned before, are NSCs. These NSCs exist in discrete regions of the adult mammalian brain where neurogenesis and oligodendrogenesis are highly regulated (Faigle and Song, 2013). The brain regions where these processes take place, *id est*, where the NSC pools can be encountered, are called neurogenic niches. In adulthood, there are two main neurogenic niches in the brain: the subventricular zone (SVZ) of the lateral ventricles, and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus (Paspala *et al*., 2011).

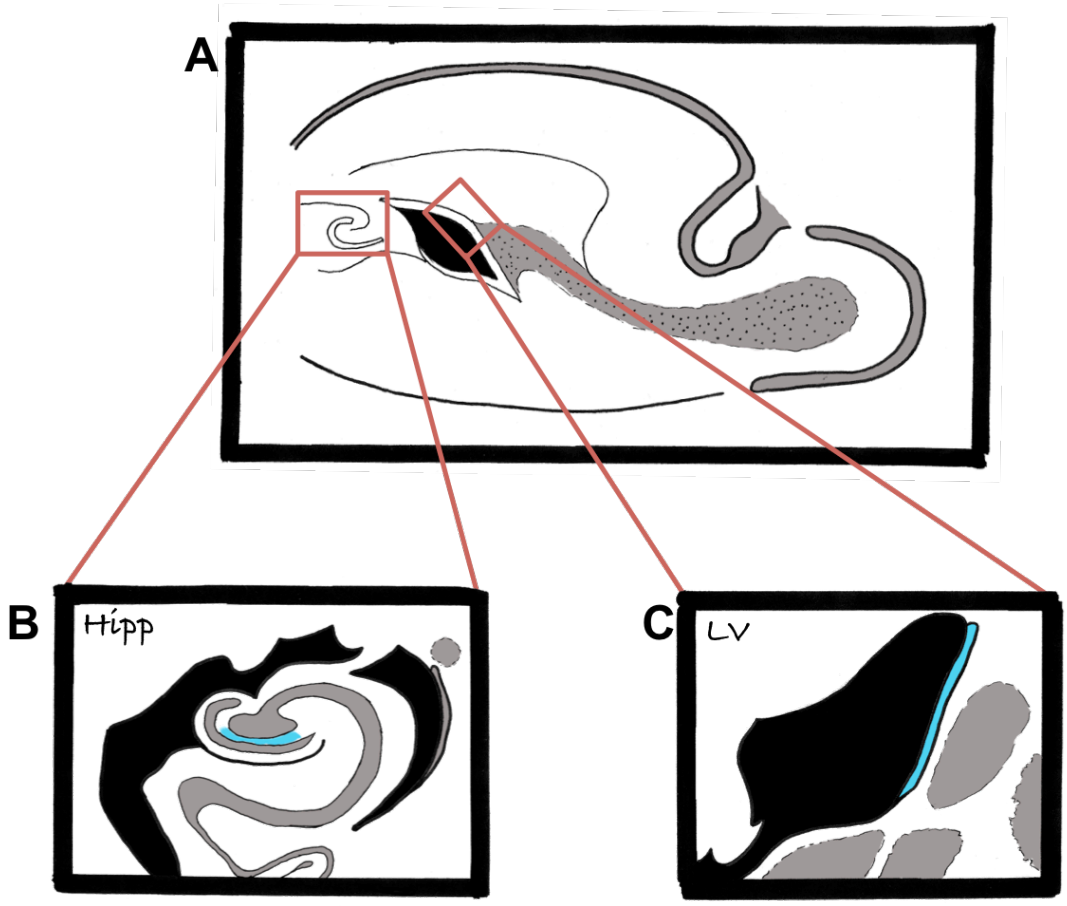

Figure 1. A schematic illustration of the adult mammalian neurogenic niches. (**A**) Parasagittal plane showing the hippocampus (Hipp) and the lateral ventricle (LV). Adult neural stem cells are primarily present in two germinal regions: the subgranular zone of the hippocampal dentate gyrus (DG, **B**) and the subventricular zone (SVZ, **C**).

In the SVZ, the NSC pool comprises type B cells (protoplasmic astrocyte-like stem cells), which are quiescent NSC that can originate C cells, which are fast dividing transient amplifying cells which give rise to type A cells (migrating neuroblasts that are constantly proliferating) (Bergström and Forsberg-Nilsson, 2012; Doetsch *et al*., 1997; García-Verdugo *et al*., 1998). The type A cells, the most common cell type in the SVZ, then form a chain towards the olfactory bulb (OB). This chain is enclosed by type B cells, while type C cells follow in close proximity to the chain (García-Verdugo *et al*., 1998). Most of type A cells will then differentiate into neuronal progenitors, migrate along the rostral migratory stream (RMS) (Kirschenbaum *et al*., 1999) to the OB, and terminally differentiate into mainly GABAergic and dopaminergic interneurons (Doetsch *et al*., 1999; Lazarini *et al*., 2014; Lois and Alvarez-Buylla, 1993). SVZ-derived oligodendrogenesis originates from a minority of B and C cells that do not follow the previously explained cellular fate. Instead, they produce OPCs, which migrate radially out of the SVZ into the surrounding cortex and white matter (Cayre *et al*., 2009; Menn *et al*., 2006; Suzuki and Goldman, 2003). Indeed, the SVZ is the source of OPCs that migrate and populate the *corpus callosum*, *striatum*, and *fimbria-fornix* in the adult mouse (Menn *et al*., 2006; Ortega *et al*., 2013). One important SVZ characteristic is that its multipotent stem cell-like precursors can be collected and originate, *in vitro* and in the presence of the epidermal growth factor (EGF) and the basic fibroblast growth factor 2 (FGF-2), neurospheres capable of self-renewal, proliferation, and differentiation in neurons, astrocytes, and oligodendrocytes (Doetsch *et al*., 1997; Gritti *et al*., 1996; Hack *et al*., 2004; Reynolds and Weiss, 1996, 1992).

It should be noted that one NSC can generate either oligodendrocytes or neurons exclusively (Ortega *et al*., 2013) and that the number of oligodendrocytes produced

by the SVZ NSC pool is significantly inferior to the number of olfactory interneurons (Menn *et al*., 2006). The relative quantity of oligodendrocytes and neurons is area dependent: while in the posterior zone of the SVZ the ratio is one oligodendrocyte to three neurons, in the rostral zone this ratio is one oligodendrocyte to thirty neurons (Menn *et al*., 2006). This ratio also changes dorsoventrally, due to environmental cues. The dorsal part of the SVZ is Wnt enriched, which favours OPC commitment (Ortega *et al*., 2013). On the other hand, the ventral part is more exposed to bone morphogenic proteins (BMP), which inhibits OPC specification (Colak *et al*., 2008).

In humans, SVZ-derived neurogenesis and oligodendrogenesis were considered controversial for a long time (Breunig *et al*., 2007). At first, there were reports that a RMS-like structure could not be identified in humans (Sanai *et al*., 2004) and that migratory neuronal precursors only existed in the first years of life, disappearing during childhood (Weickert *et al*., 2000). However, data is still contradictory, as another study was able to identify a complex structure engulfing the SVZ, RMS, and the OB in the human brain (Curtis *et al*., 2007). Moreover, it was shown that the SVZ-derived interneurons were able to integrate active neuronal circuits in the OB (Pignatelli and Belluzzi, 2010). Additionally, NSCs were isolated from the human SVZ, corroborating SVZ as a neurogenic niche (Sanai *et al*., 2004). Curiously, in humans, the number of neuroblasts along the RMS is very reduced when compared to other mammals (Wang *et al*., 2011), and SVZ-derived neurons do not seem to migrate extensively to the OB (Ernst and Frisén, 2015). In fact, evidence has been found that, in humans, SVZ-derived neuroblasts are able to migrate to the striatum, where they differentiate into interneurons (Ernst *et al*., 2014; Ernst and Frisén, 2015). As to oligodendrogenesis, SVZ activation has been witnessed in the presence of a demyelinating insult, specifically in *post-mortem* samples of patients with multiple

sclerosis (MS). In this situation, the SVZ showed an increased formation of new OPCs (Nait-Oumesmar *et al*., 2007).

1.4 MS: WHY OLIGODENDROCYTES MATTER

MS is an inflammatory neurodegenerative demyelinating disease in which the myelin sheath covering the nerve cells in the brain and spinal cord is damaged, thus disrupting the ability of neurons to communicate (Compston and Coles, 2008). The cause for this disease is still unclear, but the underlying mechanism is thought to be either autoimmune or based on oligodendrocyte failure (Nakahara *et al*., 2012). There is no known cure for MS and the life expectancy for affected individuals is 5 to 10 years lower than that of an unaffected population (Compston and Coles, 2008).

It is known that demyelinating pathologies can stimulate adult oligodendrogenesis. In fact, in mice, when a demyelinating insult occurs, OPCs, both SVZ-derived and parenchymal, proliferate and migrate to the damaged area, where they mature and originate new oligodendrocytes and, consequently, a new myelin sheath around the affected neurons (Xing *et al*., 2014). Moreover, an increase in cell density and proliferation of the SVZ has been found in *post-mortem* samples of MS patients (Nait-Oumesmar *et al*., 2007). However, this process of remyelination most of the times is not efficient. Particularly, in MS lesions, a blocked remyelination is observed – OPCs are present in the insulted zone, but fail to differentiate into oligodendrocytes (Chang *et al*., 2000; Levine and Reynolds, 1999). Thus, it is of most importance to understand this process of differentiation and create strategies to overcome the block in remyelination.

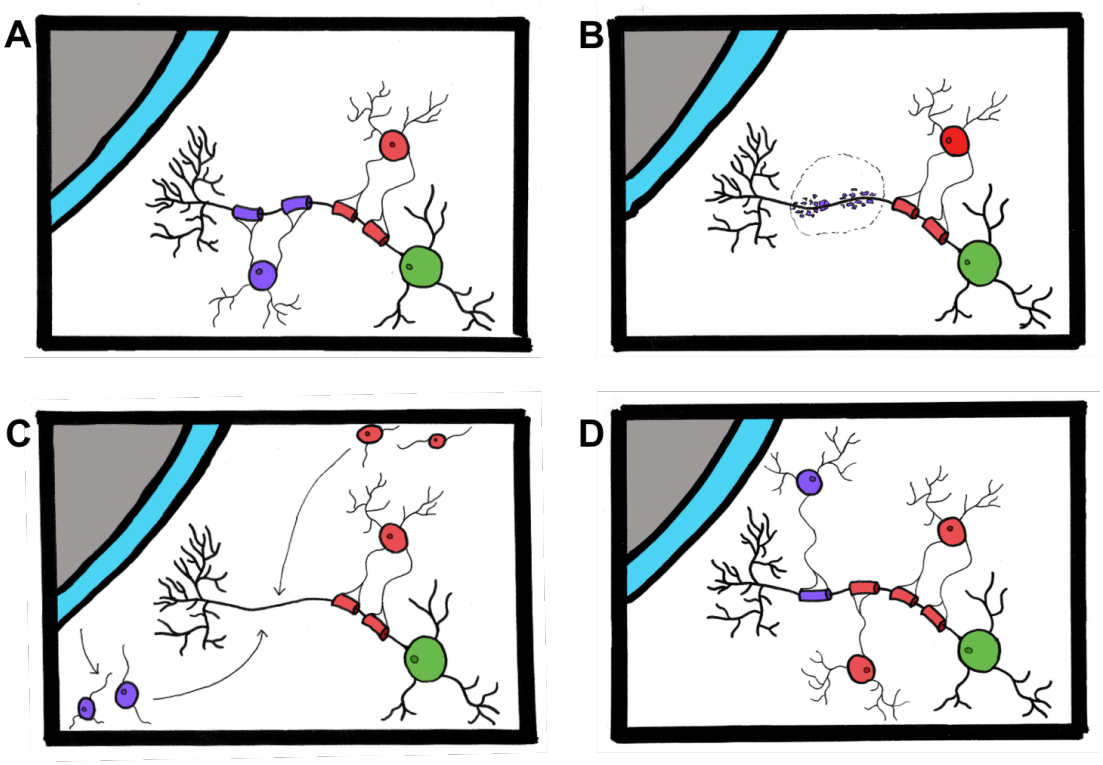

Figure 2. Oligodendrocytic response towards a demyelinating insult (Lateral ventricle – grey; SVZ – blue; neuron – green; OPCs and oligodendrocytes – purple and red). (**A**) In control conditions, oligodendrocytes form a myelin sheath around the neuronal axon. (**B**) When a demyelinating insult occurs, some oligodendrocytes are destroyed. (**C**) Both SVZ-derived and parenchymal OPCs (purple and red respectively) migrate towards the lesion zone. (**D**) In this zone, the OPCs differentiate into oligodendrocytes and restore the myelin sheath.

1.5 SVZ-DERIVED OLIGODENDROGENESIS MODULATION

Oligodendrogenesis is modulated by extrinsic and intrinsic factors. The extrinsic factors include morphogens, growth factors, and signalling molecules delivered through blood vessels or associated to the extracellular matrix. The intrinsic factors include transcription factors and epigenetic markers.

Different extrinsic factors modulate oligodendrogenesis in the adult SVZ. Evidence show that both factors secreted by blood vessels and factors connected to the extracellular matrix are capable of favouring OPC commitment (Chintawar *et al.*, 2009; Plane *et al.*, 2010). One of these factors is laminin, an element of the

extracellular matrix. A study in mice shows that the elimination of laminin α2-subunit leads to a reduction of the OPC population in the SVZ (Relucio *et al*., 2012). Other trophic factors, such as PDGF (Jackson *et al*., 2006; Rafalski *et al*., 2013) and EGF (Aguirre *et al*., 2007; Gonzalez-Perez and Alvarez-Buylla, 2011), contribute indirectly to oligodendrocyte lineage determination, through promoting OPC proliferation and maturation.

The main intrinsic factor involved in oligodendrocyte determination is the transcription factor Olig2 (El Waly *et al*., 2014). This basic helix-loop-helix (bHLH) factor is induced by sonic hedgehog (Shh) (Lu *et al*., 2002) and expressed in every stage of oligodendrocyte maturation, from precursor cell to myelinating oligodendrocyte (El Waly *et al*., 2014). In the majority of the CNS, inactivation of Olig2 during development leads to a reduction in OPCs (Ligon *et al*., 2006; Lu *et al*., 2002; Zhou and Anderson, 2002). In contrast, overexpression of Olig2 in neuroepithelium leads to enhanced OPC production in the CNS (Maire *et al*., 2010). Even in the postnatal brain, overexpression of Olig2 leads to enhanced oligodendrogenesis in the SVZ (Maire *et al*., 2010). Furthermore, the presence of Olig2 is sufficient to reprogram rat and mouse fibroblasts into induced OPCs (Najm *et al*., 2013; Yang *et al*., 2013). Although this factor is crucial to oligodendrocyte differentiation, Olig2 knockout mice are still able to produce some OPCs in the hindbrain, possibly through Olig1 compensation (Lu *et al*., 2002).

In the last few years, epigenetic modulation of oligodendrogenesis has been gaining some importance, namely regarding the modulation by microRNA and histone modifications. For instance, miRNA-219 and miRNA-338 inhibit neuronal commitment of NSCs, leading to the production of OPCs (Dugas *et al*., 2010; Zhao *et*

al., 2010). Furthermore, these two miRNA contribute to OPC differentiation into mature oligodendrocytes (Dugas *et al*., 2010; Zhao *et al*., 2010).

Histone modifications can also be important for oligodendrogenesis. For instance, it has been shown that oligodendrocyte production from NSCs, instead of other cellular fate, depends on histone deacetylases (Hdac) activity (Liu and Casaccia, 2010). Similarly, a study using Enhancer of zeste homolog 2 (Ezh2), a polycomb group protein involved in gene silencing through histone methylation, provided evidence that a higher rate of histone methylation (via Ezh2 overexpression) leads to an increase in oligodendrocyte production (Sher *et al*., 2008).

1.6 ADENOSINE

Adenosine is an endogenous purine ribonucleoside, composed by a molecule of adenine in glycosidic linkage with a ribose sugar. Adenosine plays important physiological roles in building nucleic acids; energy transfer, as adenosine triphosphate (ATP) and adenosine diphosphate (ADP); signal transduction, as cyclic adenosine monophosphate (cAMP); and neurotransmission, as a neuromodulator (Gharibi, 2013; Kumar *et al*., 2013; Nelson and Cox, 2008). Furthermore, adenosine has been shown to play important roles in sleep and arousal, cognition, memory and learning, neuroprotection, and regulation of blood pressure and heart rate (Sebastião and Ribeiro, 2009). There are four adenosine receptors: A_1, A_{2A}, A_{2B}, and A_3 (**Figure 3**) which are all G-protein-coupled receptors that affect the adenylate cyclase (AC) activity. A_1 and A_3 receptors interact with $G_{i/o}$, inhibiting the AC activity and reducing cAMP availability. On the other hand, both A_2 receptors generally interact with G_s, stimulating the AC and increasing the cellular level of cAMP (Fredholm *et al*., 2001). Although G_s-coupling is the main activated pathway in A_{2A} receptor

signalling in the periphery, in the striatum, where A_{2A} receptors are more abundant, this receptor acts via G-olfactory (G_{olf}), a G-protein more expressed in this brain area and similar to G_s in its positive modulation of AC activity (Kull *et al*., 2000). The main difference between the receptors A_{2A} and A_{2B} receptors lies in their affinity towards adenosine: A_{2A} receptor shows a higher affinity (0.1 – 1.0 μM) while A_{2B} receptor is only activated when it is exposed to 10 μM or higher concentrations of adenosine (Daly *et al*., 1983). Due to this difference in adenosine affinity, only A_{2A} receptor is activated with physiological concentrations of adenosine (Gharibi, 2013). However, it has also been shown that this receptor, A_{2A}, when coupled with G_s, desensitizes rapidly (Chern *et al*., 1995, 1993; Palmer and Stiles, 1997; Ramkumar *et al*., 1991).

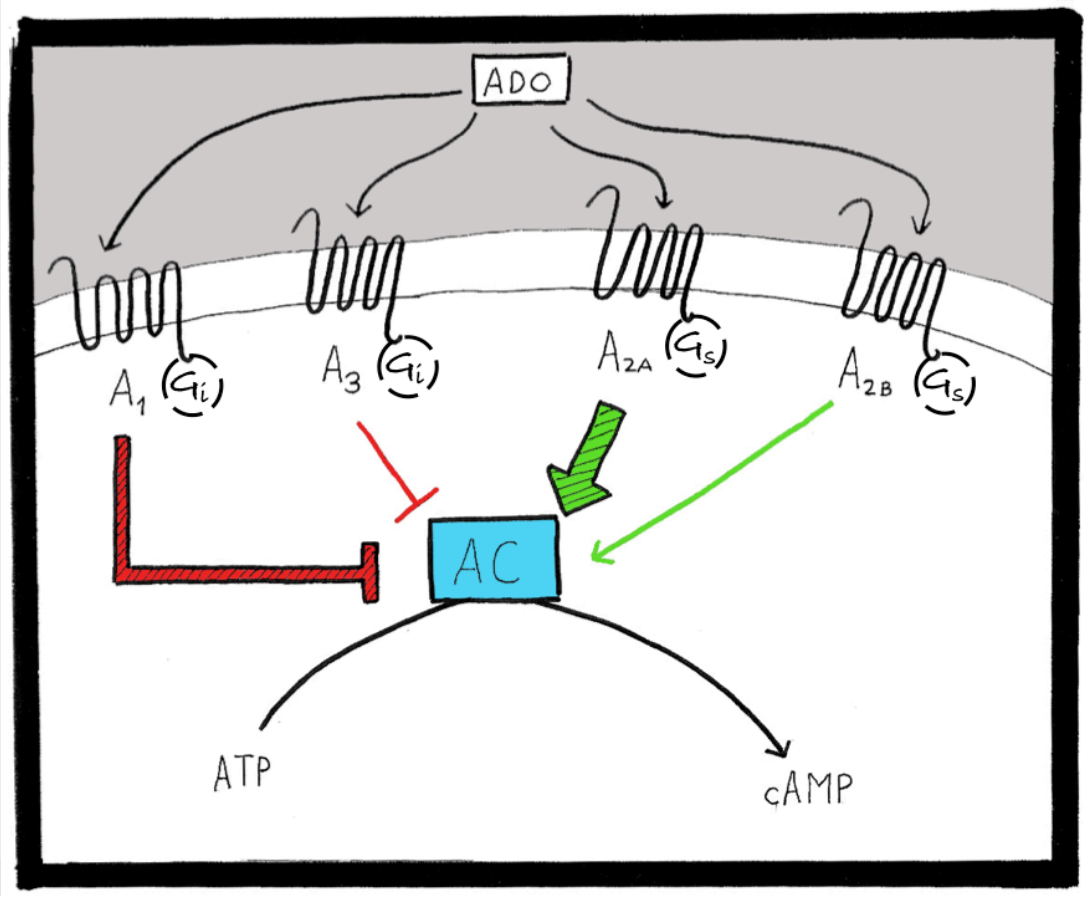

Figure 3. Schematic representation of the four G-protein coupled adenosine receptors and their effects on adenylate cyclase and on cAMP levels. ADO – Adenosine; AC – Adenylate cyclase.

Adenosine has been identified as the main active axon-glia signalling molecule in the CNS (Stevens *et al*., 2002). Indeed, it is known that all subtypes of adenosine receptors are expressed on different cells in the CNS, including oligodendrocytes (Agresti *et al*., 2005; Othman *et al*., 2003) and OPCs (Stevens *et al*., 2002), as well as in the peripheral nervous system (Dunn *et al*., 2001; Dunwiddie and Masino, 2001). Furthermore, it has been reported the expression of A_{2A} receptors in neurospheres obtained from the SVZ (Stafford *et al*., 2007).

A_{2A} receptors are known to activate several intracellular signalling pathways. It has been described that A_{2A} receptor activation leads to an increase in intracellular concentration of cAMP, which will activate the protein kinase A (PKA) and the phosphoinositide 3-kinase (PI3-K) (Klinger *et al*., 2002). On the other hand, A_{2A} receptor activation regulates the mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK) pathways, namely in regard to alterations in mitogenesis, differentiation, and cell survival (Luttrell, 2002; Marinissen and Gutkind, 2001). Indeed, A_{2A} receptor activation has been reported to block cell cycle progression and to decrease cell survival in human melanoma cells, via both MAPK/ERK and protein kinase C (PKC) pathways (Merighi *et al*., 2002). Other study, performed in striatal neuronal precursors cells, showed that A_{2A} receptor activation induces cell cycle arrest and promotes differentiation via MAPK/ERK and through interaction with neurotrophin receptors (Canals *et al*., 2005). All these studies point to a modulatory role of A_{2A} receptors in the initial steps of neuronal and glial differentiation.

A_{2A} receptors have been associated with both positive and deleterious physiological effects. A_{2A} receptor activation has been shown to mediate pain in peripheral sites, by inhibiting platelet aggregation and regulating blood pressure

(Ledent *et al*., 1997). A_{2A} receptor was also shown to be important to regulate the stimulant motor effect of caffeine (El Yacoubi *et al*., 2000), and to regulate the inflammatory responses (Ohta and Sitkovsky, 2001). Plus, A_{2A}R-knockout mice show disturbances in the CNS, namely decreased exploratory activity, aggressiveness, hypoalgesia, and reduced alcohol sensitivity (Chen *et al*., 2003; Ledent *et al*., 1997; Naassila *et al*., 2002); as well as higher risk of inflammation and higher production of pro-inflammatory cytokines (Haskó and Pacher, 2008; Sitkovsky *et al*., 2004). A_{2A} receptors also play an important role in regulating brain derived neurotrophic factor (BDNF) synaptic actions, namely in the modulation of synaptic transmission (Diógenes *et al*., 2004), enhancement of long-term potentiation (LTP) (Fontinha *et al*., 2008) and neuromuscular transmission (Pousinha *et al*., 2006), and even in the maintenance of normal BDNF levels in the hippocampus (Tebano *et al*., 2008). Furthermore, on the one hand, A_{2A} receptors were shown to be upregulated in the cerebral white matter of patients with secondary progressive MS, where this upregulation is correlated with higher scores in the disability scale (Rissanen *et al*., 2013). Furthermore, administration of A_{2A} receptor antagonist protected disease progression in a mouse model of experimental autoimmune encephalomyelitis (EAE), an animal model for MS (Mills *et al*., 2012). Indeed, the activation of A_{2A} receptors in neuronal and glial cells was sufficient to induce EAE in mice (Coppi *et al*., 2015; Mills *et al*., 2012). On the other hand, genetic ablation of both peripheral and central A_{2A} receptors augments brain damage and neuroinflammation in a mouse model of EAE (Yao *et al*., 2012), which suggests that regulation of neuroinflammation by A_{2A} receptors may be necessary for myelin repair in several neurodegenerative diseases (Coppi *et al*., 2015).

Moreover, at a cellular level, adenosine receptor activation has been shown to have mitogenic and anti-proliferation effects, depending on the type of cell and microenvironment (Jacobson *et al*., 1999; Stevens *et al*., 2002). In SVZ-derived neurospheres, A_{2A} receptor activation led to a decrease in frequency and proliferation of primary neurospheres (Stafford *et al*., 2007). Importantly, previous results from our lab showed that A_{2A} receptor activation has no effect on cell death, proliferation, nor neuronal differentiation in SVZ-derived neurospheres (Ribeiro *et al.*, unpublished data). Additionally, several studies focused on the effects of adenosine in OPCs, namely in migration, proliferation, and maturation (Coppi *et al*., 2013b; Stevens *et al*., 2002). Specifically, Coppi and collaborators concluded that A_{2A} receptor activation in isolated cultured OPCs inhibits inward potassium currents and OPC differentiation, without compromising cell viability or proliferation (Coppi *et al*., 2013a). The same study also refers that FGF receptors are also expressed in OPCs and, similarly to A_{2A} receptor activation, their activation inhibits OPC differentiation, by inhibiting expression of myelin components (Besnard *et al*., 1989), and contributing to OPC proliferation (Coppi *et al*., 2015). However, there are known interactions between A_{2A} receptors and growth factor receptor signalling cascades (Stafford *et al*., 2007). In fact it was shown that when there is a concomitant activation of A_{2A} and FGF receptors, the opposite effect occurs, with robust activation of the MAPK/ERK pathways. Indeed, this combined action of A_{2A} and FGF receptors has been shown to stimulate differentiation and neurite extension in PC12 cells (Flajolet *et al*., 2008). Thus, it is postulated that a cross-talk between these two receptors may occur in OPCs (Coppi *et al*., 2013a). Two other studies seem to corroborate this positive effect of adenosine on oligodendrocyte differentiation, by showing that administration of extracellular adenosine reverses growth factor-induced proliferation and inhibition of

differentiation of OPCs, thus contributing to their differentiation and maturation into oligodendrocytes, both in the presence of PDGF and FGF (Agresti *et al*., 2005; Stevens *et al*., 2002).

Taking into account all these positive and negative aspects associated with A_{2A} receptors, one can hypothesize that the consequences of A_{2A} receptor activation in oligodendrogenesis and subsequent myelination depend on the timing of stimulation and on the presence of different demyelinating pathologies (Coppi *et al*., 2015).

2 Aims

Taking into consideration that a demyelinating insult such as MS activates the SVZ niche the aim of this study was to investigate the putative role of A_{2A} receptor in inducing oligodendrogenesis derived from the SVZ. This work can be divided in two parts: first, to trace a pattern of expression of oligodendrocytic markers throughout days of cell culture; secondly, to evaluate the effect of A_{2A} receptor activation or inhibition on neural stem cell-derived oligodendrogenesis in SVZ. This line of research may be proven essential for the development of strategies to treat or prevent demyelinating disorders like MS and to gain a better understanding of the mechanisms behind oligodendrogenesis in the adult brain.

3 Methodology

3.1 ETHICS

All experiments were performed in accordance with the European Community (86/609/EEC; 2010/63/EU; 2012/707/EU).

3.2 SVZ CELL CULTURE

SVZ neurospheres were prepared from early postnatal (P1-3) Sprague-Dawley rats. After sacrificing the animal and removing its brain, SVZ fragments were dissected out from 450 μm-thick coronal brain slices, digested with 0.05% Trypsin-EDTA (Life Technologies, Carlsbad, CA, USA) in Hank's balanced saline solution (HBSS, Life Technologies), and mechanically dissociated with a P1000 pipette. The originated cell suspension was then diluted in serum-free medium (SFM), composed of Dulbecco's modified Eagle's medium/Ham's F-12 medium with glutaMAX (DMEM+GlutaMAX, Life Technologies) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin (Pen/Strep; Life Technologies), 1% B27 (Life Technologies), 20 ng/mL epidermal growth factor (EGF; Life Technologies) (proliferative conditions). The cells were then plated on uncoated Petri dishes and allowed to develop for six days in a 95% air-5% CO_2 humidified atmosphere at 37 °C.

3.3 PHARMACOLOGICAL TREATMENT

For immunocytochemistry, the 6-days-old neurospheres were adhered for 24h onto glass coverslips coated with 0.1 mg/mL poly-D-lysine (Sigma-Aldrich, St. Louis, MO, USA) in SFM without growth factors (differentiative conditions). In order to

study oligodendrocytic differentiation, the medium was then renewed and the cells grew for more 2, 4, or 7 days with or without A_{2A} receptor agonist (CGS21680, 30 nM, Tocris, Bristol, UK) and/or A_{2A} receptor antagonist (ZM241385, 50 nM, Tocris).

For the enzymatic assay, the 6-day-old neurospheres were adhered for 24h onto 6-well plates coated with 0.1 mg/mL poly-D-lysine in SFM without growth factors. The medium was then renewed and the cells grew for 7 days with or without CGS21680 (30 nM) and/or ZM241385 (50 nM).

The concentrations of A_{2A} receptor agonist and antagonist were chosen due to their selectivity as shown by Cristóvão-Ferreira and colleagues (Cristóvão-Ferreira *et al.*, 2013).

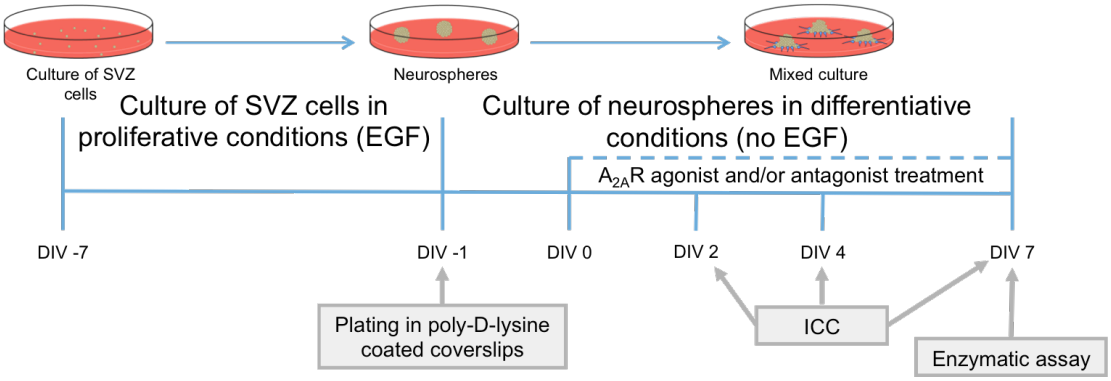

Figure 4. Schematic representation of the experimental protocol used to study oligodendrogenesis derived from SVZ cell cultures.

3.4 IMMUNOCYTOCHEMISTRY (ICC)

Cells were fixed for 30 minutes in paraformaldehyde 4% (w/v, Sigma-Aldrich) in phosphate-buffered saline (PBS), and permeabilized and blocked for non-specific binding sites for 1h30 with bovine serum albumin 3% (w/v, BSA, Sigma-Aldrich) in PBS. Cells were then incubated overnight at 4 °C with the primary antibodies (**Table 1**) and BSA 3% (w/v) in PBS. The following day, after washing the cells with PBS, they were incubated for 1h at room temperature with the appropriate secondary

antibodies in PBS. Nuclei were stained with Hoechst 33342 12 µg/mL (Life Technologies) in PBS. The final preparations were mounted using Mowiol fluorescent medium. Fluorescence images were recorded using an Axioskop 2 Plus fluorescent microscope (Carl Zeiss Inc., Göttingen, Germany). The chosen markers allowed us to follow the whole process of differentiation, from precursor cells to mature oligodendrocytes, as illustrated by **Figure 5**.

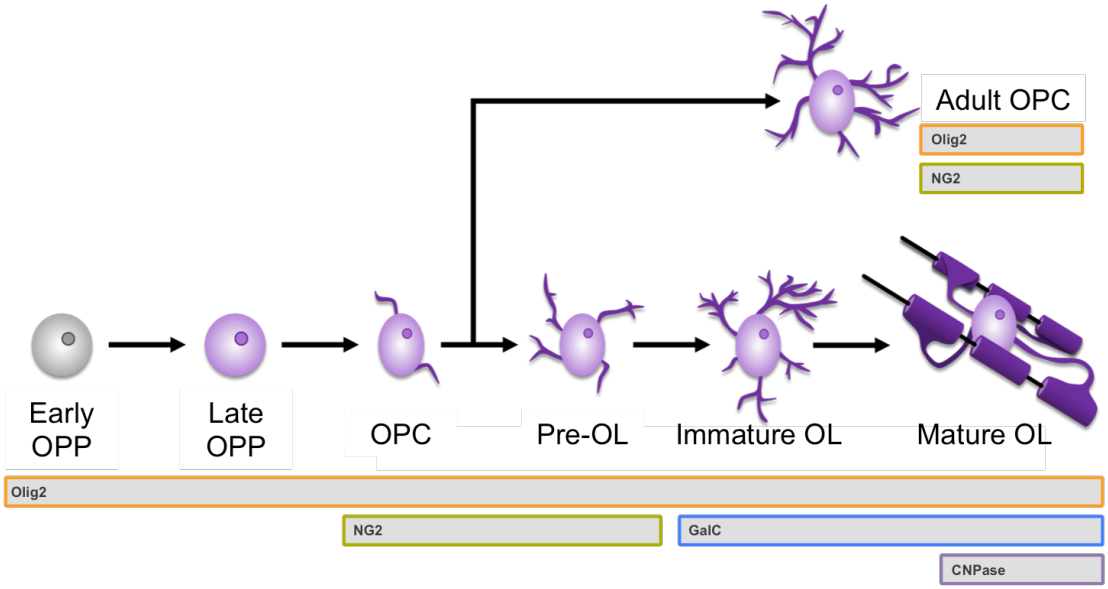

Figure 5. Schematic representation of the developmental stages of the oligodendrocytic lineage and of chosen markers to study oligodendrogenesis. The chosen markers were Olig2, a transcription factor expressed throughout the oligodendrocytic lineage; the neuron-glial antigen 2 (NG2), expressed only by OPCs, adult OPCs, and pre-oligodendrocytes; galactocerebroside (GalC), expressed by immature and mature oligodendrocytes; and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase), a myelin-related enzyme expressed by myelinating oligodendrocytes. OPP – oligodendrocyte pre-progenitor; OPC – Oligodendrocyte precursor cell; OL – Oligodendrocyte.

Table 1. List of used primary antibodies

Antigen	Company	Technique	Host	Dilution
Olig2	Millipore (AB9610)	ICC	Rabbit	1:200
NG2	Millipore (AB5320)	ICC	Rabbit	1:200
GalC	Millipore (MAB342)	ICC	Mouse	1:200

3.5 ENZYMATIC ASSAY FOR CNPASE

CNPase (2',3'-cyclic nucleotide 3'-phosphodiesterase, EC 3.1.4.37) is a myelin-related enzyme, expressed by mature myelinating oligodendrocytes (Kurihara and Tsukada, 1967). This enzyme has been used as a marker of myelination (Nishizawa *et al*., 1980), using *in vitro* either 2',3'-cyclic-AMP (Wells and Sprinkle, 1981) or 2',3'-cyclic-ß-NADP (cNADP) (Sogin, 1976) as substrate.

Accordingly, in order to study whether the NSC-derived oligodendrocytes are mature and functionally intact, CNPase enzyme activity was measured using a coupled assay with the enzyme glucose-6-phosphate dehydrogenase, following the procedure described by Lee and colleagues (Lee *et al*., 2001), with minor modifications, according to the following biochemical principle:

$$\text{2',3'-cyclic-ß-NADP} + H_2O \xrightarrow{\text{CNPase}} \text{ß-NADP} \quad (1)$$

$$\text{ß-NADP} + \text{Glucose-6-phosphate} \xrightarrow{\text{Glucose-6-phosphate dehydrogenase}} \text{6-phosphogluconate} + \text{ß-NADPH} \quad (2)$$

In reaction (1), cNADP is converted to NADP, in a reaction catalysed by CNPase. This product is then consumed in reaction (2), as a consequence of the transformation of glucose-6-phosphate into 6-phosphogluconate. The end product is NADPH, which can be measured spectrophotometrically at 340 nm and has the extinction coefficient $\varepsilon_{340\,nm}^{\mathrm{NADPH}} = 6.22\ \mathrm{cm^{-1}\ mM^{-1}}$.

Protein extracts from SVZ cells incubated with or without A_{2A} receptor agonist and/or antagonist for seven days were prepared by washing and scraping with 100 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (Merck, Kenilworth, New Jersey, United States). Afterwards, the cells were sonicated (5 times, 70% with a duty

cycle of 0.7, for 10 seconds) and centrifuged at 10000 rpm for 15 minutes, in order to isolate the cytosolic fraction. Protein concentration was measured with the Bio-Rad Protein dye reagent (Bio-Rad, Hercules, CA, USA).

For the enzymatic assay, the protein extracts (in 100 mM MES buffer pH 6.0) were added to 30 mM $MgCl_2$ (Merck), 5 mM glucose-6-phosphate (G6P, Sigma-Aldrich), and 5 U/mL glucose-6-phosphate dehydrogenase (G6PDH, Sigma-Aldrich), in a reaction volume of 1.1 mL. Protein concentration in the assay was optimized using the control extracts. Different concentrations of CNPase substrate cNADP (BioLog, Bremen, Germany; prepared in MES buffer) were used, depending on the finality of the assay. For kinetic parameters determination, the substrate concentration varied between 1 mM and 4 mM. For the specific activity determination, a concentration of 4.94 mM was used in the assay.

All reactions were initiated without the substrate cNADP, following this reaction for 3 minutes. The substrate was then added, without stopping the reaction, and this was followed for 7 more minutes. NADPH formation was measured at 340 nm at 25 °C, using the extinction coefficient $\varepsilon = 6.22\ mM^{-1}\ cm^{-1}$. All kinetic assays were performed in a diode-array spectrophotometer Agilent 8453 (Agilent, Santa Clara, CA, USA) with controlled temperature and magnetic stirring in the *cuvette* to maintain isotropic conditions during the assay. Enzyme activities were calculated through the difference between the slopes registered before and after adding the substrate to the reaction mixture. Kinetic parameters were determined by fitting rate equations to experimental initial-rate data by non-linear least squares regression: CNPase apparent parameters towards cNADP were determined assuming a single-substrate Michaelis-Menten equation for the dependence of the reaction rate on substrate concentration.

3.6 DATA ANALYSIS FOR CNPASE

For the determination of protein mass to use, NADPH formation was measured using 0 µg, 20 µg and 40 µg of SVZ-derived protein extracts and 1 mM cNADP. The maximum chosen value for protein mass was 20 µg, to insure that a linear response could be seen – this linear response will allow for correct calculations of the initial rates of the reactions.

This assay also shows that the non-enzymatic transformation of cNADP to NADPH can be ignored, considering its very low rate.

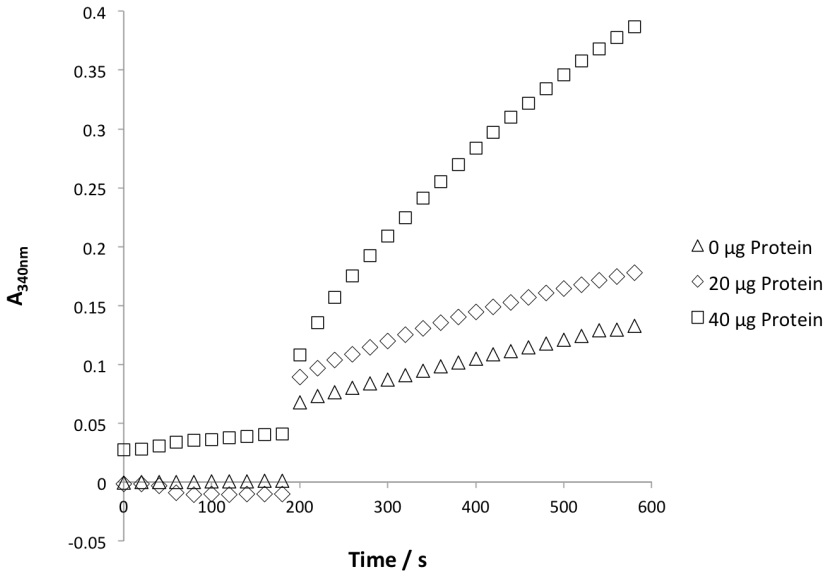

Figure 6. Enzymatic assay for the determination of protein mass to be used in activity assays. cNADP was added at 180 s, at the concentration of 1 mM.

The initial rates of the several reactions for kinetic parameters and enzymatic activity determination were calculated from the difference between the absorbance variance before and after the addition of cNADP, as demonstrated below for the 1 mM cNADP reaction in the kinetic parameters determination assay.

Slope without cNADP = $2.38 \times 10^{-5}\ s^{-1}$

Slope with cNADP = $5.15 \times 10^{-4}\ s^{-1}$

Difference = $5.15 \times 10^{-4}\ s^{-1} - 2.38 \times 10^{-5}\ s^{-1} = 4.91 \times 10^{-4}\ s^{-1}$

The initial rate of a reaction translates into the number of µmol of product formed by minute. Given that the two reactions involved are 1:1, the rate of NADPH formation is equivalent to the formation of NADP in reaction (1). Thus, measuring the formation of NADPH leads to initial rate of the reaction catalysed by CNPase. This said:

$$\Delta Abs.min^{-1} = Abs.s^{-1} \times 60 = 4.91 \times 10^{-4} \times 60 = 2.95 \times 10^{-2}\ min^{-1}$$

The law of Lambert-Beer relates absorbance with concentration through the following equation:

$$Abs = \varepsilon lc$$

Where ε is an extinction coefficient, l the path travelled by light, and c the concentration of the target molecule. Given that $\varepsilon_{340\ nm}^{\mathrm{NADPH}} = 6.22\ mM^{-1}\ cm^{-1}$ and that the *cuvette* path measured 1 cm:

$$\Delta Abs.min^{-1} = \varepsilon_{340\ nm}^{NADPH} \times l \times \Delta[NADPH].min^{-1}$$

$$2.95 \times 10^{-2} = 6.22 \times 1 \times \Delta[NADPH].min^{-1}$$

$$\Delta[NADPH].min^{-1} = 4.74 \times 10^{-6}\ M.min^{-1}$$

Now, given that $v_{cuv} = 1.1 \times 10^{-3}\ L$:

$$\Delta n(NADPH).min^{-1} = \Delta[NADPH].min^{-1} \times v_{cuv}$$

$$\Delta n(NADPH).min^{-1} = 4.74 \times 10^{-6} \times 1.1 \times 10^{-3}$$

$$\Delta n(NADPH).min^{-1} = 5.22 \times 10^{-3}\ \mu mol.min^{-1}$$

This variation of chemical quantity of NADPH per minute is, at last, the initial rate of the reaction.

3.7 STATISTICAL ANALYSIS

In all ICC experiments, measurements were performed at the border of SVZ neurospheres, where migrating cells form a pseudo-monolayer of cells. In every independent experiment, each condition was measured in three different coverslips.

Data are expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using one-way analysis of variance followed by Bonferroni's multiple comparison test, with $p < 0.05$ considered to represent statistical significance. All data were analysed in Prism GraphPad software.

4 Results

4.1 THE NUMBER OF OLIGODENDROCYTES DERIVED FROM SVZ NEUROSPHERES IS DEPENDENT ON THE DAYS IN CULTURE

The number of oligodendrocytes derived from SVZ neurospheres was determined at different time points in control cultures.

It was observed that the number of Olig2-positive cells, an oligodendrocytic marker expressed in all phases of oligodendrocyte maturation, shows no significant differences from 2 to 7 DIV (n= 4–7; 2 DIV: 3.821 ± 0.8878 % (% of total cells); 4 DIV: 4.671 ± 1.512 %; 7 DIV: 5.251 ± 0.4609 %).

Interestingly, the number of NG2-positive cells, a marker expressed in OPCs, pre-oligodendrocytes and adult OPCs, is increased from 2.895% ± 1.015 (% of total cells) at 2 DIV to 5.174 ± 1.439% at 4 DIV and to 8.499 ± 2.332% at 7 DIV (n= 4–6; 2 DIV vs. 4 DIV: ns; 2 DIV vs. 7 DIV: $p < 0.001$; 4 DIV vs. 7 DIV: $p < 0.05$).

Moreover, the number of GalC-positive cells, a marker expressed by mature and immature oligodendrocytes, has a significant increase from 1.258 ± 0.6441% (% of total cells) at 2 DIV to 3.391 ± 1.435% at 4 DIV and to 3.608 ± 0.4806% at 7 DIV (n= 6–7; 2 DIV vs. 4 DIV: $p < 0.01$; 2 DIV vs. 7 DIV: $p < 0.001$; 4 DIV vs. 7 DIV: ns).

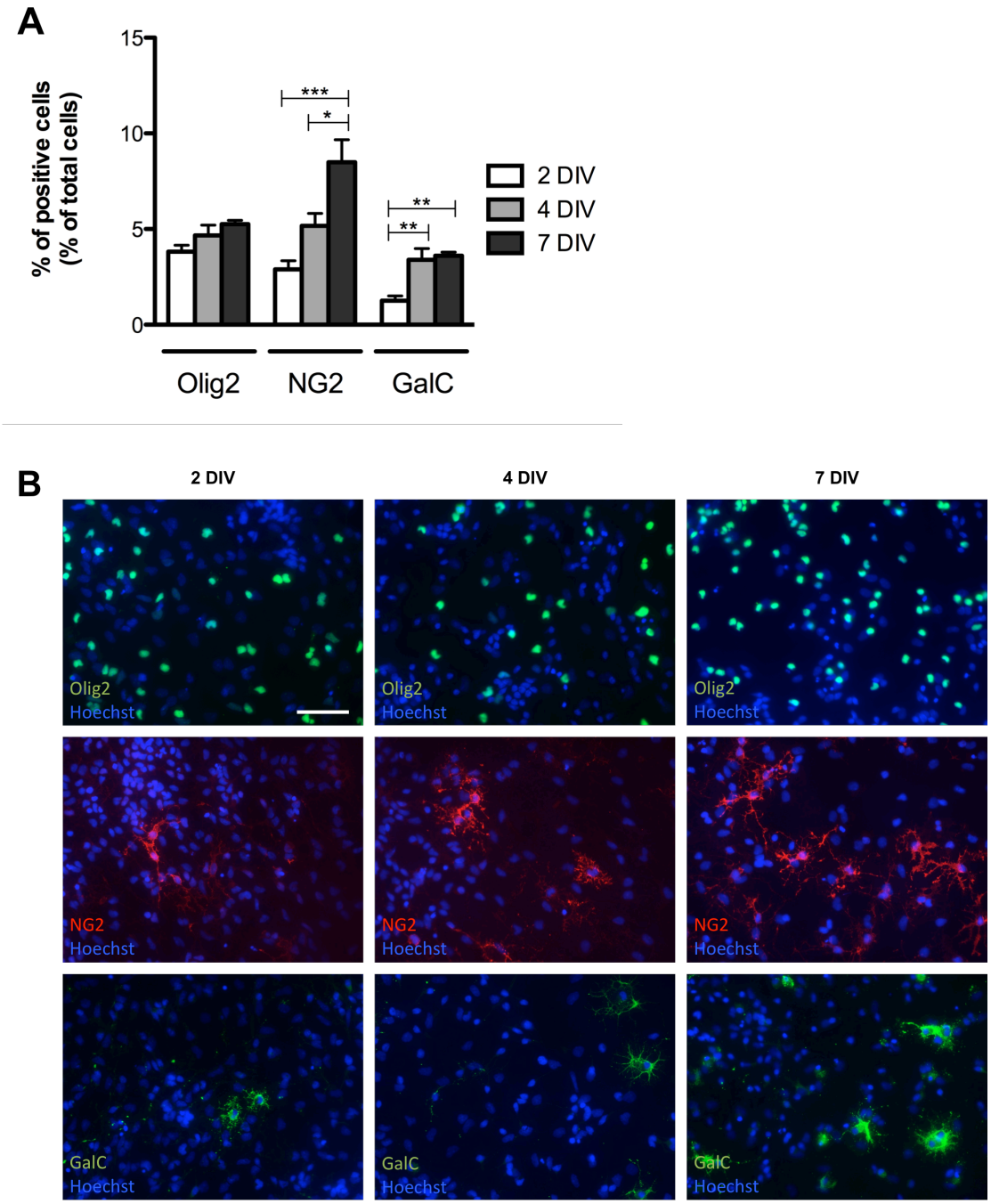

Figure 7. The number of oligodendrocytes derived from SVZ neurospheres is dependent on the days in culture. (**A**) Bar graph depicts the number of Olig2, NG2, and GalC-positive cells at 2, 4 and 7 DIV in control conditions (no pharmacological treatment with CGS21680 or ZM241385) expressed as percentage of total counted cells. N=4–8. *p<0.05, **p<0.01, ***p<0.001 using one-way ANOVA with Bonferroni's multiple comparison test. Data are expressed as mean ± SEM. (**B**) Representative fluorescent microscopy images of Olig2- (green nuclei), NG2- (red), and GalC- (green) positive cells and Hoechst 33342 staining (blue nuclei) at 2, 4 and 7 DIV. Scale bar = 50 µm.

4.2 A_{2A} RECEPTOR ACTIVATION HAS NO EFFECT ON CELL DEATH NOR PROLIFERATION

Preliminary data from our group has shown that although A_{2A} receptor activation stimulates neurogenesis from DG neural stem/progenitor cells cultures, it has no effect on SVZ cultures (Ribeiro *et al.*, unpublished data).

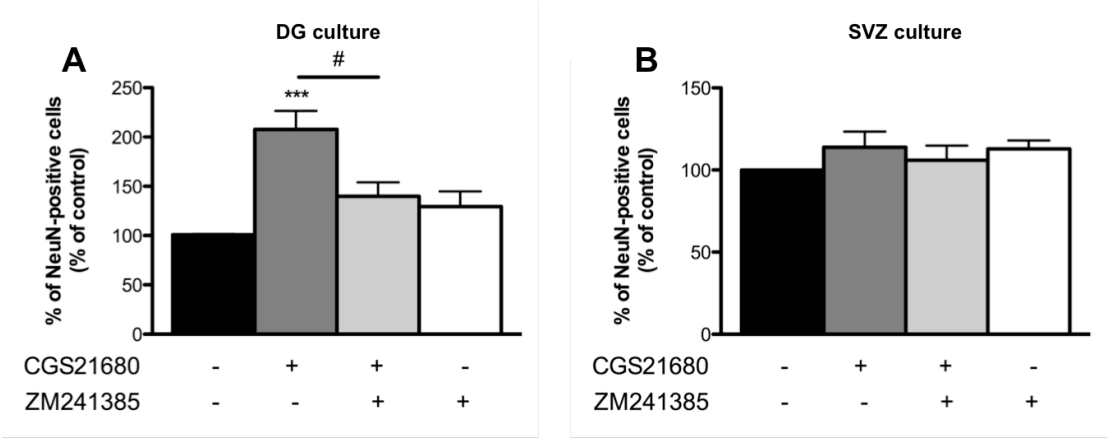

Figure 8. A_{2A} receptor activation promotes DG neuronal differentiation (**A**) while it has no effect on SVZ neurogenesis (**B**). Bar graphs depict the number of neuronal nuclei (NeuN)-positive cells in cultures maintained for 7 days in the absence (control) or in the presence of the A_{2A} receptor agonist (CGS21680, 30 nM) and/or antagonist (ZM241385, 50 nM). Values were normalized to the control mean for each experiment and are represented as the mean ± SEM. Control was set to 100%. N=6–10. ***$p < 0.001$, by ANOVA using Bonferroni's post test for comparison with control; #$p < 0.05$ using Bonferroni's post test for comparison with CGS21680.

Furthermore, in the SVZ, pharmacological treatment with A_{2A} receptor agonist (CGS21680) has no effect in cell death (measured by propidium iodide, PI, a polar compound that crosses damaged membranes, i.e., marks dead or dying cells) nor in cell proliferation (measured by bromodeoxyuridine, BrdU, a synthetic nucleoside, analogue of thymidine, which is incorporated in proliferating cells) (Ribeiro *et al.*, unpublished data).

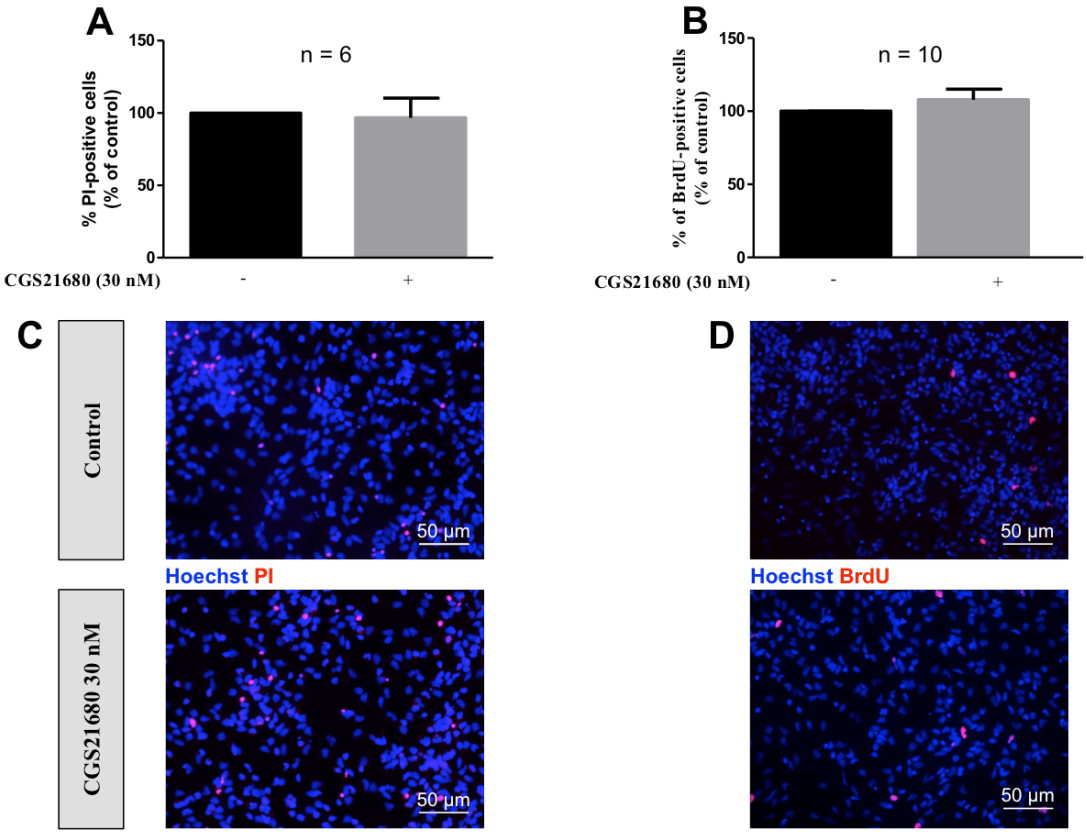

Figure 9. A_{2A} receptor activation does not induce cell death nor cell proliferation in SVZ stem/progenitor cell cultures. Bar graph depicts the percentages of propidium iodide (PI)-stained nuclei (**A**) and 5-bromo-2-deoxyuridine (BrdU)-positive cells (**B**) in cultures maintained for 24h in the absence (control) or in the presence of the A_{2A} receptor agonist (CGS21680, 30 nM). PI (3 µg/mL: labels dead/dying cells. BrdU (10 µM): an analogue of the thymidine nucleotide, labels cells that went through S-phase. Values were normalized to the control mean for each experiment and are represented as the mean ± SEM. Control was set to 100%. Representative fluorescence photos of PI (**C**) and BrdU (**D**)-positive cells. PI, BrdU (in red) and Hoechst 33342 staining (in blue) in SVZ cells in control and in CGS21680 treated cultures.

4.3 A_{2A} RECEPTOR ACTIVATION PROMOTES AN INCREASE IN THE NUMBER OF OLIG2-POSITIVE CELLS AT 2 AND 4 DIV

The main aim of this work was to study the modulatory role of A_{2A} receptor activation in SVZ-derived oligodendrogenesis. As so, all chosen markers were studied individually, in cells treated with A_{2A} receptor agonist (CGS21680, 30nM) and/or antagonist (ZM241385, 50 nM).

It was observed that A_{2A} receptor activation with CGS21680 (30 nM) promoted an increase in the number of Olig2-positive cells at 2 DIV (127.6 ± 4.624%) and 4 DIV (129.4 ± 25.66%) (n= 5–7; 2 DIV: Ctrl vs. CGS: $p < 0.01$; 4 DIV: Ctrl vs. CGS: $p < 0.05$). This effect was blocked by co-incubation of CGS21680 (30 nM) with the A_{2A} receptor antagonist ZM241385 (50 nM) (2 DIV: 98.98 ± 15.26 %; 4 DIV: 92.49 ± 9.687 %), while ZM241385 (50 nM) had no effect of its own (2 DIV: 98.67 ± 13.73 %; 4 DIV: 100.8 ± 20.92 %), when comparing with control cultures (2 DIV and 4 DIV: 100.0 ± 0.01%).

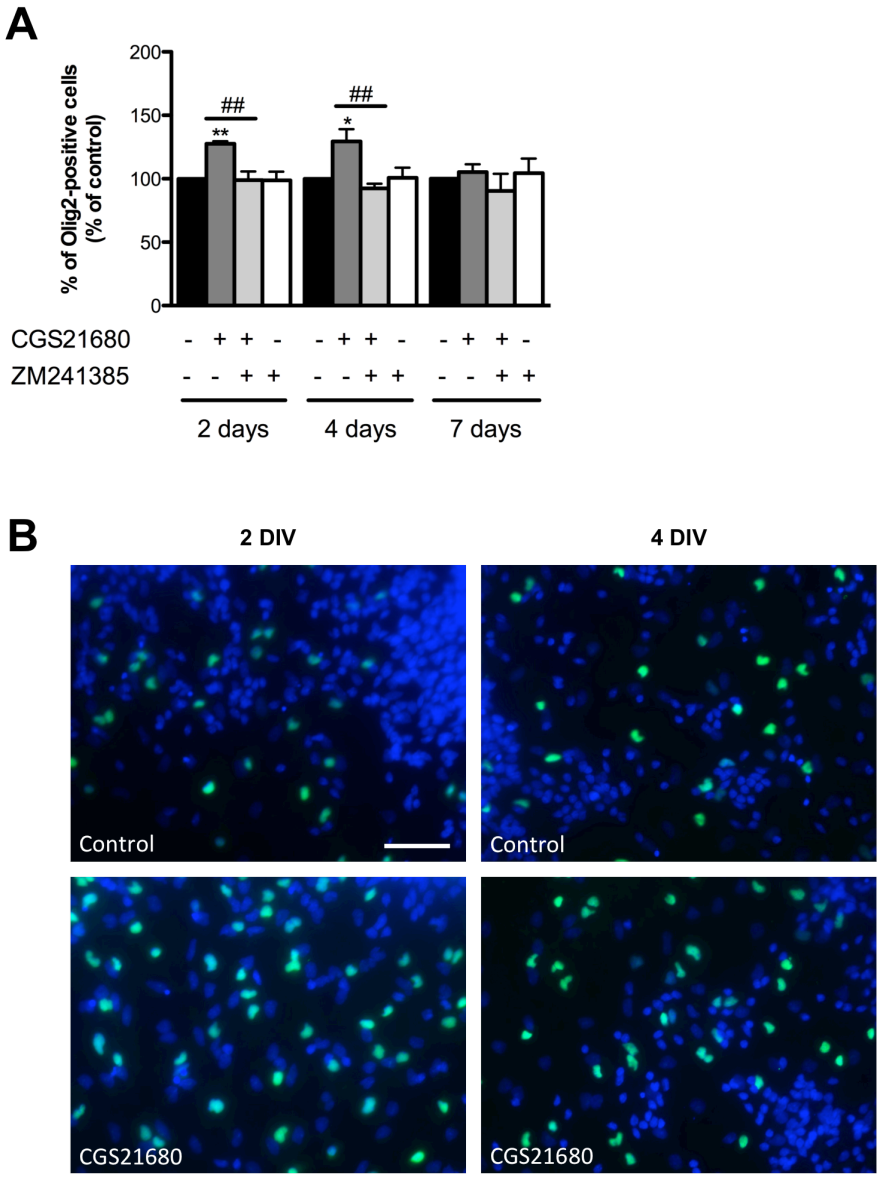

Figure 10. A_{2A} receptor activation promotes an increase in the number of Olig2-positive cells at 2 and 4 DIV. (**A**) Percentage of Olig2-positive cells at 2, 4, and 7 days in vitro. Values were normalized to the control mean for each experiment and are represented as the mean ± SEM. Control was set to 100%. N=5-8. *p<0.05, **p<0.01 using one-way ANOVA with Bonferroni's multiple comparison test, for comparison with the respective control; ##p<0.01 using one-way ANOVA with Bonferroni's multiple comparison test, for comparison with the respective CGS21680. (**B**) Representative fluorescent microscopy images of Olig2-positive cells (green) and Hoechst 33342 staining (blue nuclei) in control cultures and in cultures exposed to CGS21680 (30 nM) for 2 and 4 DIV. Scale bar = 50 μm.

4.4 A_{2A} RECEPTOR ACTIVATION PROMOTES AN INCREASE IN THE NUMBER OF NG2-POSITIVE CELLS AT 2 AND 7 DIV

NG2 is a more specific oligodendrocytic marker, being only expressed in intermediate stage of differentiation (OPC, pre-oligodendrocyte, and adult OPC). When incubated with the A_{2A} receptor agonist (CGS21680, 30 nM), the number of NG2-positive cells increased at 2 DIV (156.6 ± 30.23%) and 7 DIV (126.6 ± 25.68%) when comparing with control cultures (n= 4–6; 2 DIV: Ctrl vs. CGS: $p < 0.01$; 7 DIV: Ctrl vs. CGS: $p < 0.05$). This effect was prevented by incubation with both CGS21680 (30 nM) and the A_{2A} receptor antagonist (ZM241385, 50 nM) (2 DIV: 104.6 ± 12.86 %; 7 DIV: 86.63 ± 13.15 %), while incubation with ZM241385 (50 nM) alone had no effect (2 DIV: 104.6 ± 11.85 %; 7 DIV: 103.4 ± 14.37 %) when compared to control cultures (2 DIV: 100.0 ± 0.01 %; 7 DIV: 100.0 ± 0.002 %).

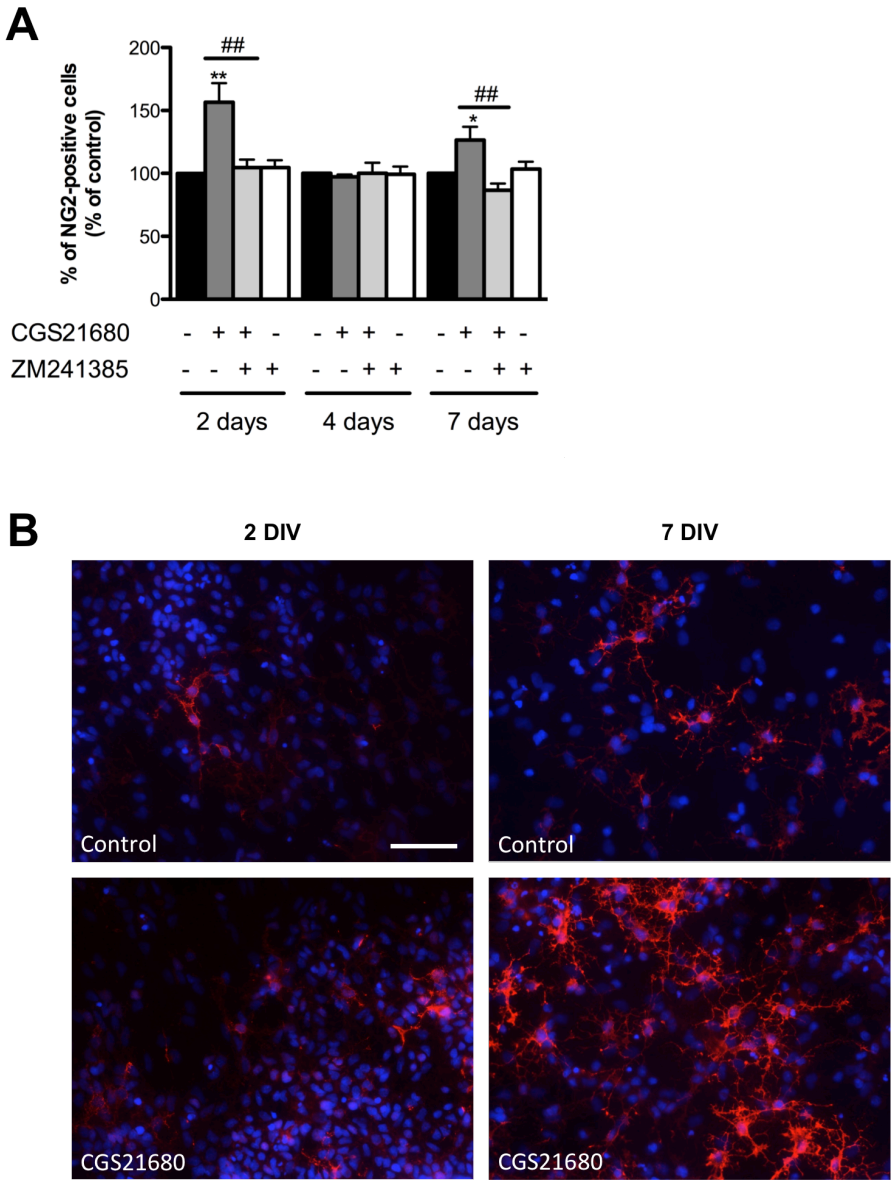

Figure 11. A_{2A} receptor activation promotes an increase in the number of NG2-positive cells at 2 and 7 DIV. (**A**) Percentage of NG2-positive cells at 2, 4, and 7 days in vitro. Values were normalized to the control mean for each experiment and are represented as the mean ± SEM. Control was set to 100%. N=4-5. *p<0.05, **p<0.01 using one-way ANOVA with Bonferroni's multiple comparison test, for comparison with the respective control; ##p<0.01 using one-way ANOVA with Bonferroni's multiple comparison test, for comparison with the respective CGS21680. (**B**) Representative fluorescent microscopy images of NG2-positive cells (red) and Hoechst 33342 staining (blue nuclei) in control cultures and in cultures exposed to CGS21680 (30 nM) for 2 and 7 DIV. Scale bar = 50 µm.

4.5 A_{2A} RECEPTOR ACTIVATION PROMOTES AN INCREASE IN THE NUMBER OF GALC-POSITIVE CELLS AT 2 AND 7 DIV

GalC, a marker expressed by both mature and immature oligodendrocytes, expression was also studied. Similarly to NG2, the number of GalC-positive cells is increased at two isolated peaks when incubated with the A_{2A} receptor agonist (CGS21680, 30 nM), at 2 DIV (153.7 ± 49.51%) and at 7 DIV (133.0 ± 13.07%) (n= 6–7; 2 DIV: Ctrl vs. CGS: $p < 0.05$; 7 DIV: Ctrl vs. CGS: $p < 0.001$). ZM241385 (50 nM) had no effect when incubated alone (2 DIV: 105.2 ± 8.206 %; 7 DIV: 98.00 ± 16.56 %), while the effect mediated by CGS21680 was blocked by co-incubation with the A_{2A} receptor antagonist (ZM241385, 50 nM) (2 DIV: 94.74 ± 22.26 %; 7 DIV: 106.2 ± 12.26 %) as compared to control cultures (2 DIV: 100.0 ± 0,01 %; 7 DIV: 100.0 ± 0.00 %).

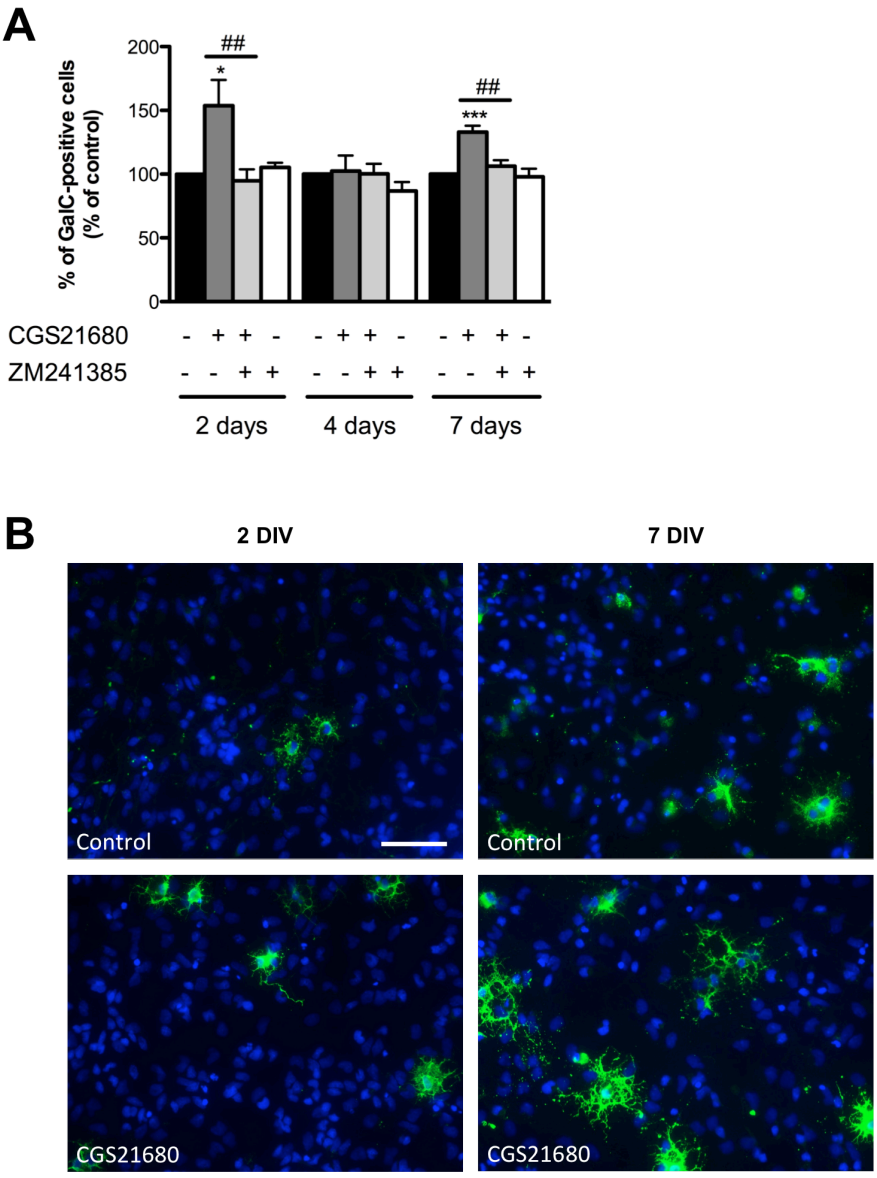

Figure 12. A_{2A} receptor activation promotes an increase in the number of GalC-positive cells at 2 and 7 DIV. (**A**) Percentage of GalC-positive cells at 2, 4, and 7 days in vitro. Values were normalized to the control mean for each experiment and are represented as the mean ± SEM. Control was set to 100%. N=6-7. *p<0.05, ***p<0.001 using one-way ANOVA with Bonferroni's multiple comparison test, for comparison with the respective control; ##p<0.01 using one-way ANOVA with Bonferroni's multiple comparison test, for comparison with the respective CGS21680. (**B**) Representative fluorescent microscopy images of GalC-positive cells (green) and Hoechst 33342 staining (blue nuclei) in control cultures and in cultures exposed to CGS21680 (30 nM) for 2 and 7 DIV. Scale bar = 50 µm.

4.6 CNPase shows increased enzymatic activity in cells treated with CGS21680

An enzymatic assay to indirectly determine the maturation of oligodendrocytes was performed regarding CNPase (2',3'-cyclic nucleotide 3'-phosphodiesterase, EC 3.1.4.37), since this is a myelin-related enzyme, expressed by myelinating oligodendrocytes.

Therefore, in order to study whether the NSC-derived oligodendrocytes are mature and functionally intact, an enzymatic assay of CNPase was conducted in cells grown for 7 days, according to the following biochemical principle:

$$\text{2',3'-cyclic-ß-NADP} + H_2O \xrightarrow{\text{CNPase}} \text{ß-NADP} \quad (1)$$

$$\text{ß-NADP} + \text{Glucose-6-phosphate} \xrightarrow{\text{Glucose-6-phosphate dehydrogenase}} \text{6-phosphogluconate} + \text{ß-NADPH} \quad (2)$$

In the first place, the kinetic parameters of CNPase were determined, as shown in **Figure 13**, using 20 µg of protein extract incubated with different cNADP concentrations, from 1 mM to 4 mM.

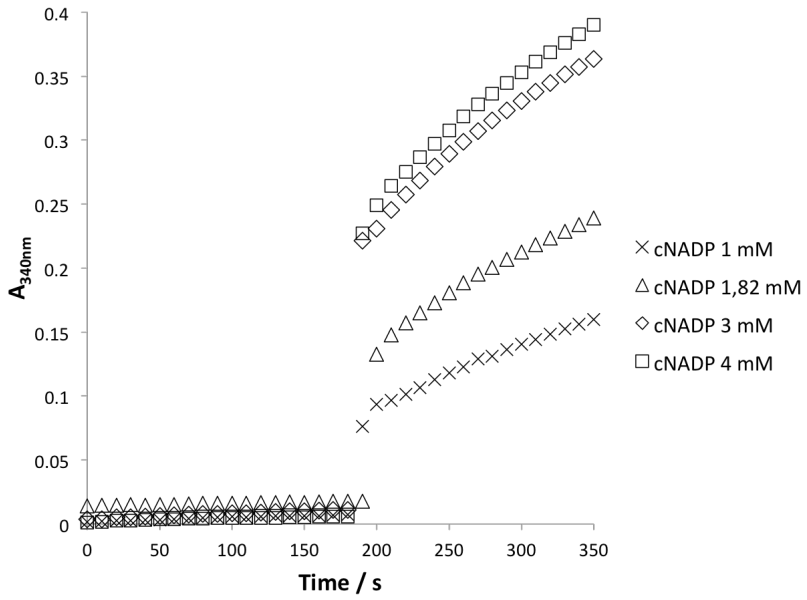

Figure 13. Enzymatic assay for the determination of CNPase kinetic parameters. 20 µg of extract were used. cNADP was added at 180 - 190 s, at several concentrations (1 mM; 1.82 mM; 3 mM; 4 mM). Initial rates were calculated taking into account the difference in slopes before and after adding cNADP.

The initial rates of the reaction catalysed by CNPase, calculated from the difference between the absorbance variance before and after the addition of the substrate (cNADP), were then determined (**Table 2**).

Table 2. Initial rates of the reaction catalysed by CNPase with different concentrations of cNADP.

[cNADP] (mM)	**v_i (µmol.min^{-1})**
1	5.22×10^{-3}
1.82	7.82×10^{-3}
3	1.05×10^{-2}
4	1.11×10^{-2}

Assuming this enzyme follows Michaelis-Menten kinetics, its kinetic parameters — K_m and V_{max} — were calculated through a hyperbolic regression of the initial rates vs. concentration of cNADP, as shown by **Figure 14**.

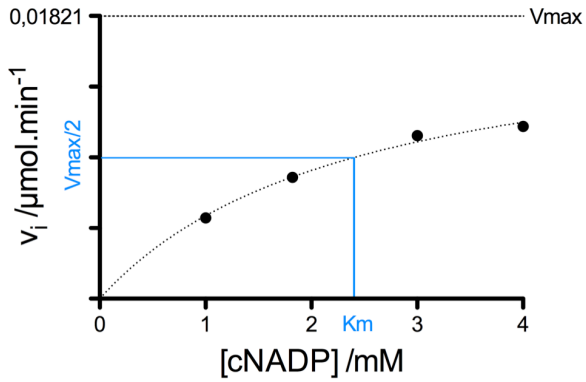

Figure 14. Hyperbolic regression of the initial rates vs. concentration of cNADP.

With this hyperbolic regression, the calculated kinetic parameters are:

$K_m = 2.404 \pm 0.4577$ mM

$V_{max} = 0.01821 \pm 0.001648$ µmol.min^{-1}

The value obtained for K_m falls into the values available in literature, which vary between 0.47 mM (Sprinkle, 1989) and 3.7 mM (Wells and Sprinkle, 1981).

With the kinetic parameters calculated, the following step was comparing the initial rates of protein extracts that were incubated during culture procedure with or without A_{2A} receptor agonist (CGS21680, 30 nM) and/or antagonist (ZM241385, 50 nM). For this, an assay using 20 µg of protein extract and 1 mM of cNADP was conducted.

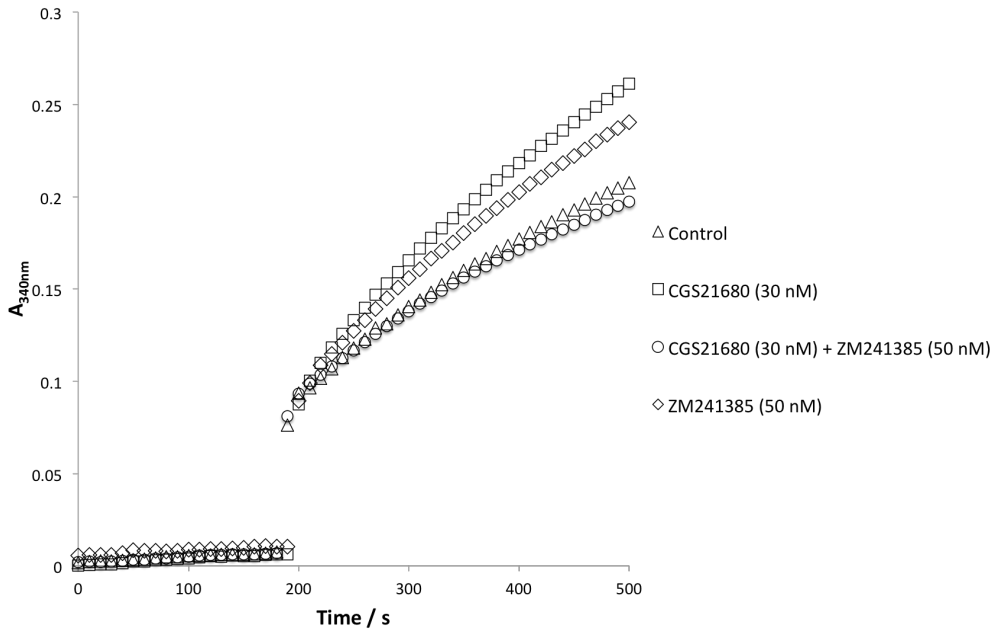

Figure 15. A_{2A} receptor activation leads to an increase in CNPase activity at 7 DIV, as shown by an enzymatic assay for the determination of initial rates of reaction in the presence of A_{2A} receptor agonist (CGS21680, 30 nM) and/or antagonist (ZM241385, 50 nM). 20 µg of extract were used. 1 mM cNADP was added at 180 - 200 s. Initial rates were calculated taking into account the difference in slopes before and after adding cNADP.

Performing the data analysis as presented in the methods section, it is possible to obtain the initial rates of the four reactions (**Table 3**).

Table 3. Initial rates for CNPase in untreated cells and in cells treated with CGS21680 (30 nM) and/or ZM241385 (50 nM) for 7 days.

Condition	v_i (µmol.min^{-1})	v_i (% of control)
Control	4.53×10^{-3}	100
CGS21680 (30 nM)	5.08×10^{-3}	112.0
CGS21680 (30 nM) + ZM241385 (50 nM)	4.61×10^{-3}	101.7
ZM241385 (50 nM)	4.54×10^{-3}	100.1

Finally, an assay was performed with excess substrate (4.94 mM cNADP), to determine CNPase specific activity in control condition and in the presence of A_{2A} receptor agonist.

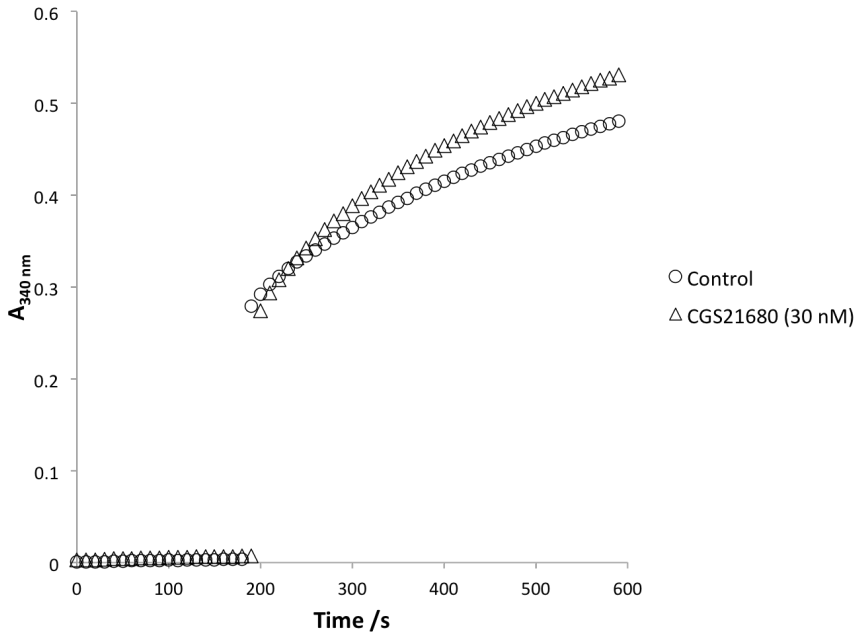

Figure 16. A_{2A} receptor activation promotes an increase in CNPase specific activity at 7 DIV. 10 µg of extract were used. 4.94 mM cNADP (excess substrate) was added at 180 - 190 s. Initial rates were calculated taking into account the difference in slopes before and after adding cNADP.

For CNPase, 1 U is described as the amount of enzyme that catalyses the conversion of 1 μmol of substrate per minute. Since the reactions involved are all 1:1, it can be concluded that the formation of NADPH is equivalent to the consumption of cNADP. Furthermore, knowing that the reaction volume was 1113.61 μL and the protein mass used was 10 μg, the specific activities were calculated. These results suggest that A_{2A} receptor activation leads to an increase in the specific activity of CNPase (**Table 4**).

Table 4. Specific activities for CNPase, in untreated cells and cells treated with CGS21680 (30 nM) for 7 days.

Condition	**Specific activity ($U.mL^{-1}$)**
Control	6.13×10^{-4}
CGS21680 (30 nM)	8.04×10^{-4}

5 Discussion

This work shows that A_{2A} receptor activation promotes an increase in oligodendrogenesis derived from SVZ NSCs. In fact, this investigation aimed to study oligodendrocyte generation from the SVZ NSC pool and analyse the modulatory role of A_{2A} receptor activation on SVZ-derived oligodendrogenesis *in vitro*.

Regarding the first objective, different types of cells of the oligodendrocytic lineage were observed along the time in culture.

It was not observed a change in the number of Olig2-positive cells throughout the days *in vitro*. The oligodendrocytic lineage marker Olig2 is a transcription factor present during all stages of oligodendrocytic differentiation, from early precursor cells to mature oligodendrocytes (Buffo *et al*., 2005; Rivers *et al*., 2008). However, a small portion of cells expressing this marker can also originate Olig2-positive astrocytes (Buffo *et al*., 2005). Given that all precursor and mature cells of the oligodendrocytic lineage express Olig2, the fact that the number of positive cells is similar at different time points was indeed expected (Rivers *et al*., 2008). The "jump" from neurospheres to cells in differentiation starts to happen at -1 DIV (as shown by **Figure 4**), when the neurospheres are first exposed to differentiative conditions (removal of growth factors and plating in a substrate) (Cavaliere *et al*., 2012). Moreover, since the first time point shown corresponds to 2 DIV, the cells derived from the neurospheres already had time to commit into the different cellular fates – neuronal, glial or oligodendrocytic (Jensen and Parmar, 2006; Torrado *et al*., 2014). Therefore, at two days *in vitro*, early OPCs, expressing Olig2, are already present in the culture and will differentiate into oligodendrocytes, also expressing Olig2, which enlightens why the number of Olig2-positive cells may not change along differentiation (Rivers *et al*., 2008).

Interestingly, the number of NG2- and GalC-positive cells varies during culture differentiation. Indeed, the number of NG2-positive cells, an antigen expressed by OPCs, pre-oligodendrocytes, and adult OPCs, increased throughout the days in culture, specially accentuated in the interval between four and seven days *in vitro*. Concomitantly, it was observed an increase during culture differentiation in the number of GalC-positive cells, a marker of immature and mature oligodendrocytes, with highest variation between two and four days *in vitro*. Since NG2 and GalC are markers expressed during certain stages of oligodendrocyte differentiation, the cell populations grow in culture as the NSC further differentiate.

Analysing both markers, it can be concluded that NSCs originate Olig2-positive cells that will mature into OPCs and oligodendrocytes throughout culture differentiation. In fact, it was already shown that SVZ NSCs in culture can originate OPCs and oligodendrocytes (Cavaliere *et al*., 2012). It should be noted that these results point to the existence of quiescent OPCs that do not maturate immediately into oligodendrocytes. This is also to be expected, due to the known existence, in the adult brain, of adult OPCs that remain in this undifferentiated stage until a demyelinating insult signals them to differentiate (Rafalski *et al*., 2013; Zhang and Chopp, 2013). Therefore, at seven days *in vitro*, the neurosphere-derived mixed culture has undifferentiated precursors, as well as oligodendrocytes, as well as cells of the other lineages (neuronal and astroglial).

Finding drugs able to modulate oligodendrogenesis is of high importance in several demyelinating disorders. Therefore, our results showing that A_{2A} receptor activation has a positive modulatory role in SVZ-derived oligodendrogenesis is significant. Specifically, it was shown an increase in the number of Olig2-positive

cells at two and four days *in vitro*; and NG2- and GalC-positive cells at two and seven days *in vitro*.

Moreover, by using an enzymatic assay of CNPase, a myelin-related enzyme, it was shown that A_{2A} receptor activation leads to the existence of more cells expressing this enzyme. This relation with myelin has been described since 1967 (Kurihara and Tsukada, 1967), but the enzyme's physiological function remained unknown for decades. Only now is the scientific community getting closer to ascertain its role in the brain. Recently, CNPase has been linked to local adenosine production in traumatic brain injury and to a possible regulatory function in mitochondrial membrane permeabilization, complementing its connection to myelination (Raasakka and Kursula, 2014).

Regardless of the controversy about CNPase function, this enzyme has been used as a marker of myelination (Nishizawa *et al*., 1980), using *in vitro* either 2',3'-cyclic-AMP (Wells and Sprinkle, 1981) or 2',3'-cyclic-ß-NADP (cNADP) (Sogin, 1976) as substrate.

Consequently, these results suggest that A_{2A} receptor activation induces an increase in mature myelinating oligodendrocytes since only these cells express CNPase (Girolamo *et al*., 2010; Trapp *et al*., 1988). Most importantly, not only there are more mature myelinating oligodendrocytes when cells are incubated with A_{2A} receptor agonist but they are functionally intact and capable of producing myelin.

These results are in accordance with several published papers. In the first place, the existence of A_{2A} receptors in neurospheres derived from the SVZ was already shown by Stafford and collaborators (Stafford *et al*., 2007). Furthermore, the same group showed that A_{2A} receptor activation in this neurosphere model leads to decreased

proliferation of SVZ-derived primary neurospheres (Stafford *et al*., 2007), which indirectly may imply that A_{2A} receptor activation favours NSC differentiation. Additionally, a study in mice with EAE showed that genetic inactivation of A_{2A} receptors aggravates brain damage. Since this is model of a demyelinating disorder, this could also indirectly imply that A_{2A} receptor activation is necessary for the formation of new oligodendrocytes that could ameliorate brain damage associated with EAE (Yao *et al*., 2012).

Other authors also showed that A_{2A} receptors are expressed in oligodendrocytes (Agresti *et al*., 2005; Othman *et al*., 2003) and OPCs (Dunn *et al*., 2001; Dunwiddie and Masino, 2001). Moreover, Coppi and collaborators showed that A_{2A} receptor activation inhibits OPC differentiation in *in vitro* parenchymal OPCs, thus inhibiting oligodendrocyte maturation (Coppi *et al*., 2013a). It should however be noted that Coppi and collaborators used isolated cortical OPCs. We used SVZ-derived neurospheres, a culture comprising neural stem cells, neuronal and glial precursors, neurons, astrocytes, and oligodendrocytes. Given the diversity of cell types found in SVZ-derived neurospheres, more complex cell-to-cell interactions can occur, possibly impacting the final outcome of A_{2A} receptor activation.

On the other hand, interactions between A_{2A} receptors and growth factor signalling cascades have been described (Stafford *et al*., 2007), particularly in OPCs (Coppi *et al*., 2013a). In fact, several authors show that, while some growth factor signalling cascades have a negative effect in OPC differentiation, concomitant activation of A_{2A} receptors reverses this effect, promoting OPC differentiation and maturation into oligodendrocytes (Agresti *et al*., 2005; Stevens *et al*., 2002). In our experimental conditions, proliferative conditions were assured by the presence of EGF. Even though the medium was changed to differentiative conditions (without EGF) prior to

exposing the cells to the A_{2A} receptor agonist, the action mediated by the growth factor could promote the actions of A_{2A} receptor, leading to a combined effect of these two pathways. This could explain why the fold change of oligodendrocytes in the first GalC expression peak is higher than in the second: in the first there would be a combined effect of A_{2A} receptor activation and growth factor signalling cascades while, in the second, A_{2A} receptor activation would act alone, leading to the formation of less oligodendrocytes.

There could be several explanations regarding the mechanism by which A_{2A} receptor activation modulates oligodendrogenesis derived from SVZ NSCs. For instance, these results could imply that A_{2A} receptor activation not only exacerbates the first "wave" of oligodendrocytic precursors (derived from the aforementioned "jump" that happens at day zero when cells are first exposed to differentiative conditions and A_{2A} receptor agonist) but also continues to force NSC into the oligodendrocytic fate during the first days of incubation.

The change that A_{2A} receptor activation brings to the number of Olig2-positive cells shows that A_{2A} receptor activation not only increases the number of cells committed to the oligodendrocytic fate seen in control conditions, but also leads to the formation of more early OPCs in the first days of the experiment – until four days *in vitro*. At seven days *in vitro*, however, the percentage of Olig2-positive cells is similar to control, probably due to desensitation of the A_{2A} receptors (Mundell and Kelly, 2011). However, this does not mean that the cell types present at 7 DIV in the control and A_{2A} receptor treated cultures are the same in both conditions. Indeed, the cell types are different, as the NG2 and GalC expression patterns show. Regarding these

markers, there are two isolated moments of increased expression – one at 2 DIV and other at 7 DIV.

How can we relate the peaks in Olig2-, NG2-, and GalC-positive cells? In the first place, A_{2A} receptor activation increases oligodendrocytic lineage commitment in the first days in culture, when the cells are first incubated with A_{2A} receptor agonist. Consequently, at 2 days *in vitro*, there already is a mixed culture of early oligodendrocytic precursors (Olig2-positive cells), OPCs (NG2-positive cells), and oligodendrocytes (GalC-positive). However, not all NSC differentiate at the same rate. Indeed, some may take longer to differentiate and commit to a certain cell lineage. This could explain that, at 4 days *in vitro*, there is still an increased number of Olig2-positive cells, but not of OPCs or oligodendrocytes. These more differentiated cells only appear at seven days *in vitro*, derived from the Olig2-positive cells increased at 4 days in vitro.

This theory assumes that A_{2A} receptor activation only affects NSCs. Another possible explanation for these results takes into consideration that OPCs and other precursor cells also express A_{2A} receptors. In this scenario, the results can be explained as consequence of the activation of A_{2A} receptors in different cell types in the first days of experiment. In this light, activation of NSCs will lead to the increased expression of Olig2, NG2 and GalC at 2 DIV. However, the second peak in NG2 and GalC expression will have a different origin: the OPCs generated at day two can originate the oligodendrocytes at day seven. The new peak in NG2 expression originates from NSCs that take a longer time to differentiate.

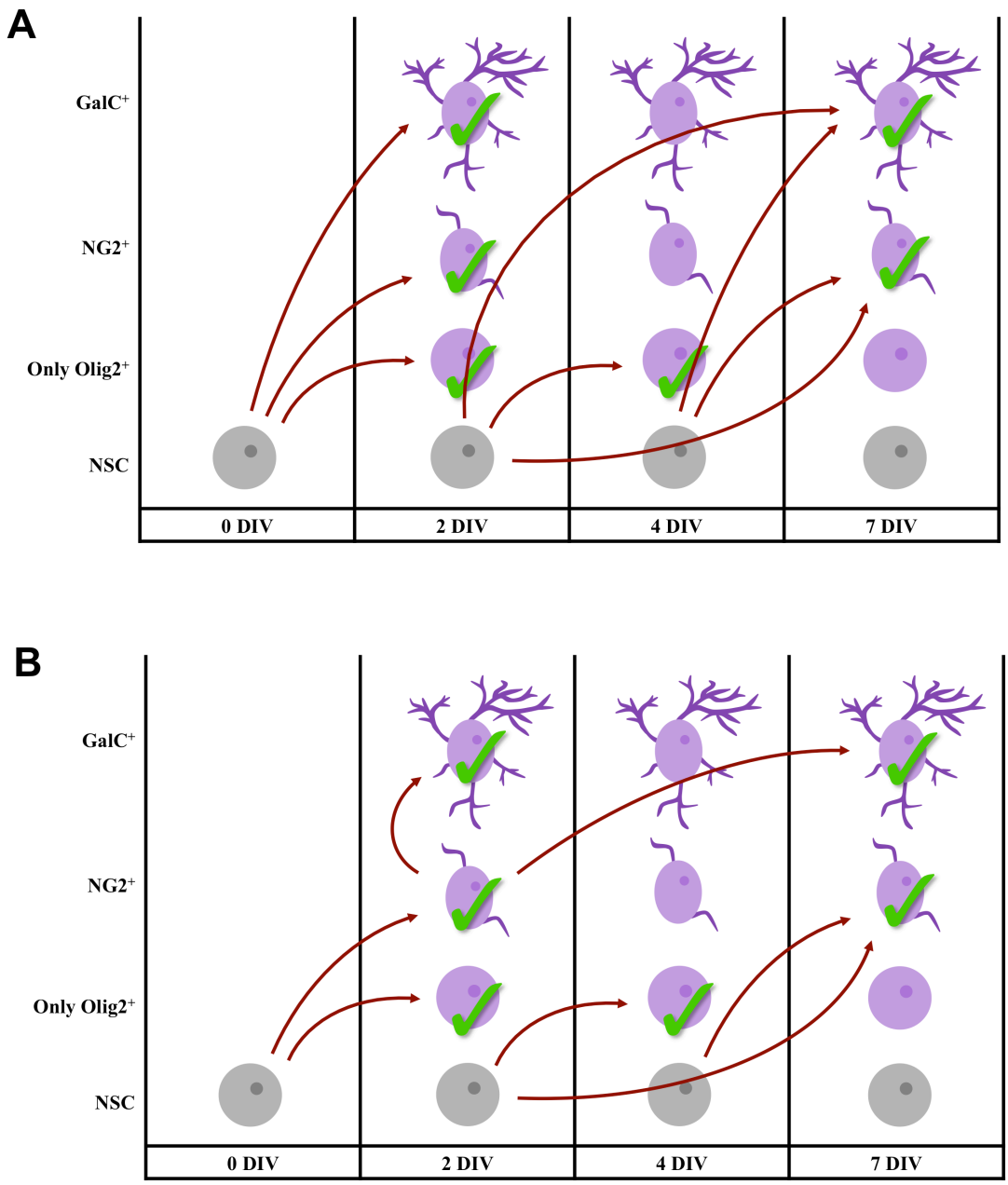

Figure 17. Schematic representation of proposed mechanisms for A_{2A} receptor modulation of SVZ-derived oligodendrogenesis. (**A**) A_{2A} receptor activation promotes differentiation of NSC at different rates. (**B**) A_{2A} receptor activation affects different cell types, such as NSCs and OPCs.

There are far too many variables to accurately predict what is happening in the culture. Different cells take different time to differentiate, A_{2A} receptors are expressed with different densities in different cell types, and the microenvironmental cues can greatly differ from cell to cell. This leads to the conclusion that further work would and will be necessary to understand the mechanism of this A_{2A} receptor-induced enhanced oligodendrogenesis. However, our data points to a relevant role of A_{2A} receptors in inducing oligodendrogenesis from SVZ NSCs which can be important for future brain therapies regarding demyelinating disorders.

6 Main conclusions

The work presented in this thesis is, to our knowledge, the first to explore the modulatory role of A_{2A} receptor activation in SVZ-derived oligodendrogenesis. Our results show that A_{2A} receptor activation leads to an increase in the number of Olig2-positive cells at 2 and 4 days *in vitro* and an increase in the number of NG2- and GalC-positive cells at 2 and 7 days *in vitro*, as shown by **Figure 18**. This means that, when A_{2A} receptors are activated, there is more production of oligodendrocytes in the SVZ NSC pool. Furthermore, through an enzymatic assay, we were able to confirm not only the formation of mature oligodendrocytes, but that these oligodendrocytes were functionally intact and capable of producing myelin. These findings are important for future therapeutic approaches for demyelinating disorders, such as MS.

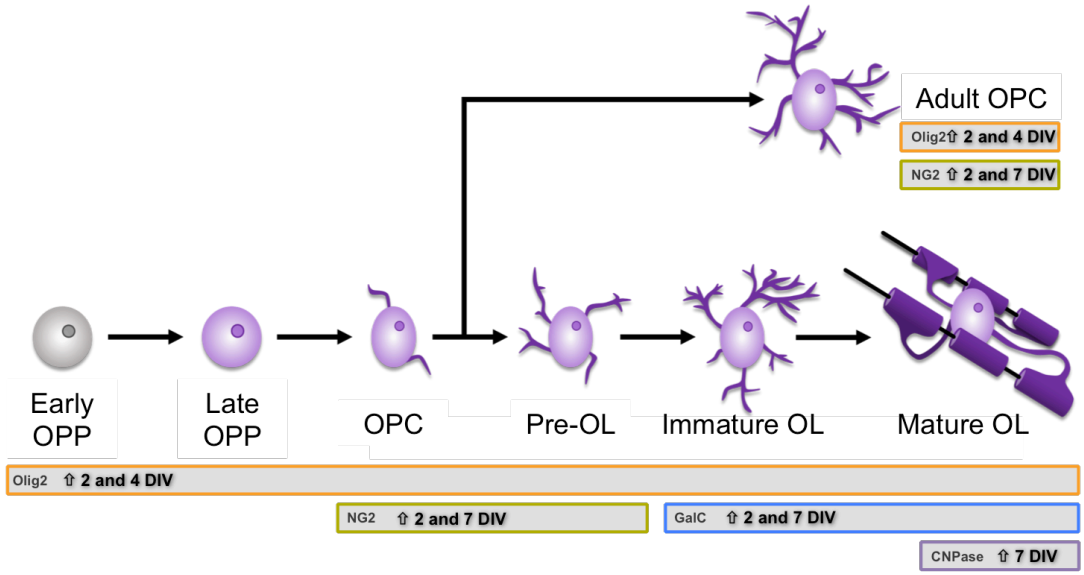

Figure 18. Schematic representation of the main conclusions. A_{2A} receptor activation promotes an increase in the number of Olig2-positive cells at 2 and 4 DIV, while it promotes an increase in the number of NG2 and GalC-positive cells at 2 and7 DIV. CNPase also shows increased enzymatic activity at 7 DIV. OPP –oligodendrocyte pre-progenitor; OPC – Oligodendrocyte precursor cell; OL – Oligodendrocyte.

However, in order to understand the mechanism behind A_{2A} receptor-mediated oligodendrogenesis, further work is necessary. This should focus on studying the effect of A_{2A} receptor activation in isolated NSCs, OPCs, and oligodendrocytes. Also, studies should be conducted to identify and understand the intracellular pathway responsible for this effect.

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8 References

Agresti, C., Meomartini, M.E., Amadio, S., Ambrosini, E., Serafini, B., Franchini, L., Volonté, C., Aloisi, F., Visentin, S., 2005. Metabotropic P2 receptor activation regulates oligodendrocyte progenitor migration and development. Glia 50, 132–144. doi:10.1002/glia.20160

Aguirre, A., Dupree, J.L., Mangin, J.M., Gallo, V., 2007. A functional role for EGFR signaling in myelination and remyelination. Nat. Neurosci. 10, 990–1002. doi:10.1038/nn1938

Alenzi, F.Q.B., Bahkali, A.H., 2014. Stem cells: Biology and clinical potential. Afr. J. Biotechnol. 10, 19929–19940. doi:10.4314/ajb.v10i86.

Back, S.A., Luo, N.L., Borenstein, N.S., Levine, J.M., Volpe, J.J., Kinney, H.C., 2001. Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury. J. Neurosci. Off. J. Soc. Neurosci. 21, 1302–1312.

Baracskay, K.L., Kidd, G.J., Miller, R.H., Trapp, B.D., 2007. NG2-positive cells generate A2B5-positive oligodendrocyte precursor cells. Glia 55, 1001–1010. doi:10.1002/glia.20519

Barateiro, A., Fernandes, A., 2014. Temporal oligodendrocyte lineage progression: in vitro models of proliferation, differentiation and myelination. Biochim. Biophys. Acta 1843, 1917–1929. doi:10.1016/j.bbamcr.2014.04.018

Baumann, N., Pham-Dinh, D., 2001. Biology of Oligodendrocyte and Myelin in the Mammalian Central Nervous System. Physiol. Rev. 81, 871–927.

Bergles, D.E., Jabs, R., Steinhäuser, C., 2010. Neuron-glia synapses in the brain. Brain Res. Rev. 63, 130–137. doi:10.1016/j.brainresrev.2009.12.003

Bergström, T., Forsberg-Nilsson, K., 2012. Neural stem cells: brain building blocks and beyond. Ups. J. Med. Sci. 117, 132–142. doi:10.3109/03009734.2012.665096

Besnard, F., Perraud, F., Sensenbrenner, M., Labourdette, G., 1989. Effects of acidic and basic fibroblast growth factors on proliferation and maturation of cultured rat oligodendrocytes. Int. J. Dev. Neurosci. Off. J. Int. Soc. Dev. Neurosci. 7, 401–409.

Breunig, J.J., Arellano, J.I., Macklis, J.D., Rakic, P., 2007. Everything that glitters isn't gold: a critical review of postnatal neural precursor analyses. Cell Stem Cell 1, 612–627. doi:10.1016/j.stem.2007.11.008

Buffo, A., Vosko, M.R., Ertürk, D., Hamann, G.F., Jucker, M., Rowitch, D., Götz, M., 2005. Expression pattern of the transcription factor Olig2 in response to brain injuries: Implications for neuronal repair. Proc. Natl. Acad. Sci. U. S. A. 102, 18183–18188. doi:10.1073/pnas.0506535102

Calver, A.R., Hall, A.C., Yu, W.P., Walsh, F.S., Heath, J.K., Betsholtz, C., Richardson, W.D., 1998. Oligodendrocyte population dynamics and the role of PDGF in vivo. Neuron 20, 869–882.

Canals, M., Angulo, E., Casadó, V., Canela, E.I., Mallol, J., Viñals, F., Staines, W., Tinner, B., Hillion, J., Agnati, L., Fuxe, K., Ferré, S., Lluis, C., Franco, R., 2005. Molecular mechanisms involved in the adenosine A and A receptor-induced neuronal differentiation in neuroblastoma cells and striatal primary cultures. J. Neurochem. 92, 337–348. doi:10.1111/j.1471-4159.2004.02856.x

Casarosa, S., Zasso, J., Conti, L., 2013. Systems for ex-vivo Isolation and Culturing of Neural Stem Cells, in: Bonfanti, L. (Ed.), Neural Stem Cells - New Perspectives. InTech.

Cavaliere, F., Urra, O., Alberdi, E., Matute, C., 2012. Oligodendrocyte differentiation from adult multipotent stem cells is modulated by glutamate. Cell Death Dis. 3, e268. doi:10.1038/cddis.2011.144

Cayre, M., Canoll, P., Goldman, J.E., 2009. Cell migration in the normal and pathological postnatal mammalian brain. Prog. Neurobiol. 88, 41–63. doi:10.1016/j.pneurobio.2009.02.001

Chang, A., Nishiyama, A., Peterson, J., Prineas, J., Trapp, B.D., 2000. NG2-positive oligodendrocyte progenitor cells in adult human brain and multiple sclerosis lesions. J. Neurosci. Off. J. Soc. Neurosci. 20, 6404–6412.

Chen, J.-F., Moratalla, R., Yu, L., Martín, A.B., Xu, K., Bastia, E., Hackett, E., Alberti, I., Schwarzschild, M.A., 2003. Inactivation of adenosine A2A receptors selectively attenuates amphetamine-induced behavioral sensitization. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 28, 1086–1095. doi:10.1038/sj.npp.1300152

Chern, Y., Chiou, J.Y., Lai, H.L., Tsai, M.H., 1995. Regulation of adenylyl cyclase type VI activity during desensitization of the A2a adenosine receptor-mediated cyclic AMP response: role for protein phosphatase 2A. Mol. Pharmacol. 48, 1–8.

Chern, Y., Lai, H.L., Fong, J.C., Liang, Y., 1993. Multiple mechanisms for desensitization of A2a adenosine receptor-mediated cAMP elevation in rat pheochromocytoma PC12 cells. Mol. Pharmacol. 44, 950–958.

Chintawar, S., Cayrol, R., Antel, J., Pandolfo, M., Prat, A., 2009. Blood-brain barrier promotes differentiation of human fetal neural precursor cells. Stem Cells Dayt. Ohio 27, 838–846. doi:10.1002/stem.25

Colak, D., Mori, T., Brill, M.S., Pfeifer, A., Falk, S., Deng, C., Monteiro, R., Mummery, C., Sommer, L., Götz, M., 2008. Adult neurogenesis requires Smad4-mediated bone morphogenic protein signaling in stem cells. J. Neurosci. Off. J. Soc. Neurosci. 28, 434–446. doi:10.1523/JNEUROSCI.4374-07.2008

Compston, A., Coles, A., 2008. Multiple sclerosis. The Lancet 372, 1502–1517. doi:10.1016/S0140-6736(08)61620-7

Coppi, E., Cellai, L., Maraula, G., Dettori, I., Melani, A., Pugliese, A.M., Pedata, F., 2015. Role of adenosine in oligodendrocyte precursor maturation. Front. Cell. Neurosci. 9, 155. doi:10.3389/fncel.2015.00155

Coppi, E., Cellai, L., Maraula, G., Pugliese, A.M., Pedata, F., 2013a. Adenosine A_2A receptors inhibit delayed rectifier potassium currents and cell differentiation in primary purified oligodendrocyte cultures. Neuropharmacology 73, 301–310. doi:10.1016/j.neuropharm.2013.05.035

Coppi, E., Maraula, G., Fumagalli, M., Failli, P., Cellai, L., Bonfanti, E., Mazzoni, L., Coppini, R., Abbracchio, M.P., Pedata, F., Pugliese, A.M., 2013b. UDP-glucose enhances outward K(+) currents necessary for cell differentiation and stimulates cell migration by activating the GPR17 receptor in oligodendrocyte precursors. Glia 61, 1155–1171. doi:10.1002/glia.22506

Cristóvão-Ferreira, S., Navarro, G., Brugarolas, M., Pérez-Capote, K., Vaz, S.H., Fattorini, G., Conti, F., Lluis, C., Ribeiro, J.A., McCormick, P.J., Casadó, V., Franco, R., Sebastião, A.M., 2013. A1R-A2AR heteromers coupled to Gs and G i/0 proteins modulate GABA transport into astrocytes. Purinergic Signal. 9, 433–449. doi:10.1007/s11302-013-9364-5

Curtis, M.A., Kam, M., Nannmark, U., Anderson, M.F., Axell, M.Z., Wikkelso, C., Holtås, S., van Roon-Mom, W.M.C., Björk-Eriksson, T., Nordborg, C., Frisén, J., Dragunow, M., Faull, R.L.M., Eriksson, P.S., 2007. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science 315, 1243–1249. doi:10.1126/science.1136281

Daly, J.W., Butts-Lamb, P., Padgett, W., 1983. Subclasses of adenosine receptors in the central nervous system: interaction with caffeine and related methylxanthines. Cell. Mol. Neurobiol. 3, 69–80.

Dawson, M.R.L., Polito, A., Levine, J.M., Reynolds, R., 2003. NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. Mol. Cell. Neurosci. 24, 476–488.

Dimou, L., Simon, C., Kirchhoff, F., Takebayashi, H., Götz, M., 2008. Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. J. Neurosci. Off. J. Soc. Neurosci. 28, 10434–10442. doi:10.1523/JNEUROSCI.2831-08.2008

Diógenes, M.J., Fernandes, C.C., Sebastião, A.M., Ribeiro, J.A., 2004. Activation of adenosine A2A receptor facilitates brain-derived neurotrophic factor modulation of synaptic transmission in hippocampal slices. J. Neurosci. Off. J. Soc. Neurosci. 24, 2905–2913. doi:10.1523/JNEUROSCI.4454-03.2004

Doetsch, F., Caillé, I., Lim, D.A., García-Verdugo, J.M., Alvarez-Buylla, A., 1999. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97, 703–716.

Doetsch, F., García-Verdugo, J.M., Alvarez-Buylla, A., 1997. Cellular Composition and Three-Dimensional Organization of the Subventricular Germinal Zone in the Adult Mammalian Brain. J. Neurosci. 17, 5046–5061.

Dugas, J.C., Cuellar, T.L., Scholze, A., Ason, B., Ibrahim, A., Emery, B., Zamanian, J.L., Foo, L.C., McManus, M.T., Barres, B.A., 2010. Dicer1 and miR-219 Are required for normal oligodendrocyte differentiation and myelination. Neuron 65, 597–611. doi:10.1016/j.neuron.2010.01.027

Dunn, P.M., Zhong, Y., Burnstock, G., 2001. P2X receptors in peripheral neurons. Prog. Neurobiol. 65, 107–134.

Dunwiddie, T.V., Masino, S.A., 2001. The role and regulation of adenosine in the central nervous system. Annu. Rev. Neurosci. 24, 31–55. doi:10.1146/annurev.neuro.24.1.31

Ellis, P., Fagan, B., Magness, S., Hutton, S., Taranova, O., Hayashi, S., McMahon, A., Rao, M., Pevny, L., 2004. SOX2, a persistent marker for multipotential neural stem cells derived from embryonic stem cells, the embryo or the adult. Dev. Neurosci. 26, 148–165.

El Waly, B., Macchi, M., Cayre, M., Durbec, P., 2014. Oligodendrogenesis in the normal and pathological central nervous system. Front. Neurosci. 8. doi:10.3389/fnins.2014.00145

El Yacoubi, M., Ledent, C., Ménard, J.F., Parmentier, M., Costentin, J., Vaugeois, J.M., 2000. The stimulant effects of caffeine on locomotor behaviour in mice are mediated through its blockade of adenosine A(2A) receptors. Br. J. Pharmacol. 129, 1465–1473. doi:10.1038/sj.bjp.0703170

Ernst, A., Alkass, K., Bernard, S., Salehpour, M., Perl, S., Tisdale, J., Possnert, G., Druid, H., Frisén, J., 2014. Neurogenesis in the striatum of the adult human brain. Cell 156, 1072–1083. doi:10.1016/j.cell.2014.01.044

Ernst, A., Frisén, J., 2015. Adult Neurogenesis in Humans- Common and Unique Traits in Mammals. PLoS Biol. 13. doi:10.1371/journal.pbio.1002045

Faigle, R., Song, H., 2013. Signaling mechanisms regulating adult neural stem cells and neurogenesis. Biochim. Biophys. Acta 1830, 2435–2448. doi:10.1016/j.bbagen.2012.09.002

Fancy, S.P.J., Zhao, C., Franklin, R.J.M., 2004. Increased expression of Nkx2.2 and Olig2 identifies reactive oligodendrocyte progenitor cells responding to demyelination in the adult CNS. Mol. Cell. Neurosci. 27, 247–254. doi:10.1016/j.mcn.2004.06.015

Flajolet, M., Wang, Z., Futter, M., Shen, W., Nuangchamnong, N., Bendor, J., Wallach, I., Nairn, A.C., Surmeier, D.J., Greengard, P., 2008. FGF acts as a co-transmitter through adenosine A(2A) receptor to regulate synaptic plasticity. Nat. Neurosci. 11, 1402–1409. doi:10.1038/nn.2216

Fontinha, B.M., Diógenes, M.J., Ribeiro, J.A., Sebastião, A.M., 2008. Enhancement of long-term potentiation by brain-derived neurotrophic factor requires adenosine A2A receptor activation by endogenous adenosine. Neuropharmacology 54, 924–933. doi:10.1016/j.neuropharm.2008.01.011

Franklin, R.J.M., Ffrench-Constant, C., 2008. Remyelination in the CNS: from biology to therapy. Nat. Rev. Neurosci. 9, 839–855. doi:10.1038/nrn2480

Fredholm, B.B., IJzerman, A.P., Jacobson, K.A., Klotz, K.N., Linden, J., 2001. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev. 53, 527–552.

García-Verdugo, J.M., Doetsch, F., Wichterle, H., Lim, D.A., Alvarez-Buylla, A., 1998. Architecture and cell types of the adult subventricular zone: In search of the stem cells. J. Neurobiol. 36, 234–248. doi:10.1002/(SICI)1097-4695(199808)36:2<234::AID-NEU10>3.0.CO;2-E

Gharibi, B., 2013. Adenosine receptor expression and function during mesenchymal stem cell differentiation and osteoblast transdifferentiation. School of Medicine, Cardiff University, Cardiff, UK.

Girolamo, F., Strippoli, M., Errede, M., Benagiano, V., Roncali, L., Ambrosi, G., Virgintino, D., 2010. Characterization of oligodendrocyte lineage precursor cells in the mouse cerebral cortex: a confocal microscopy approach to demyelinating diseases. Ital. J. Anat. Embryol. Arch. Ital. Anat. Ed Embriologia 115, 95–102.

Gonzalez-Perez, O., Alvarez-Buylla, A., 2011. Oligodendrogenesis in the subventricular zone and the role of epidermal growth factor. Brain Res. Rev. 67, 147–156. doi:10.1016/j.brainresrev.2011.01.001

Gritti, A., Parati, E.A., Cova, L., Frolichsthal, P., Galli, R., Wanke, E., Faravelli, L., Morassutti, D.J., Roisen, F., Nickel, D.D., Vescovi, A.L., 1996. Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. J. Neurosci. Off. J. Soc. Neurosci. 16, 1091–1100.

Hack, M.A., Sugimori, M., Lundberg, C., Nakafuku, M., Götz, M., 2004. Regionalization and fate specification in neurospheres: the role of Olig2 and Pax6. Mol. Cell. Neurosci. 25, 664–678. doi:10.1016/j.mcn.2003.12.012

Haskó, G., Pacher, P., 2008. A2A receptors in inflammation and injury: lessons learned from transgenic animals. J. Leukoc. Biol. 83, 447–455. doi:10.1189/jlb.0607359

Hill, R.A., Patel, K.D., Medved, J., Reiss, A.M., Nishiyama, A., 2013. NG2 cells in white matter but not gray matter proliferate in response to PDGF. J. Neurosci. Off. J. Soc. Neurosci. 33, 14558–14566. doi:10.1523/JNEUROSCI.2001-12.2013

Horner, P.J., Power, A.E., Kempermann, G., Kuhn, H.G., Palmer, T.D., Winkler, J., Thal, L.J., Gage, F.H., 2000. Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. J. Neurosci. Off. J. Soc. Neurosci. 20, 2218–2228.

Jackson, E.L., Garcia-Verdugo, J.M., Gil-Perotin, S., Roy, M., Quinones-Hinojosa, A., VandenBerg, S., Alvarez-Buylla, A., 2006. PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. Neuron 51, 187–199. doi:10.1016/j.neuron.2006.06.012

Jacobson, K.A., Hoffmann, C., Cattabeni, F., Abbracchio, M.P., 1999. Adenosine-induced cell death: evidence for receptor-mediated signalling. Apoptosis Int. J. Program. Cell Death 4, 197–211.

Jakovcevski, I., Filipovic, R., Mo, Z., Rakic, S., Zecevic, N., 2009. Oligodendrocyte development and the onset of myelination in the human fetal brain. Front. Neuroanat. 3, 5. doi:10.3389/neuro.05.005.2009

Jakovcevski, I., Zecevic, N., 2005. Sequence of oligodendrocyte development in the human fetal telencephalon. Glia 49, 480–491. doi:10.1002/glia.20134

Jensen, J.B., Parmar, M., 2006. Strengths and limitations of the neurosphere culture system. Mol. Neurobiol. 34, 153–161. doi:10.1385/MN:34:3:153

Káradóttir, R., Hamilton, N.B., Bakiri, Y., Attwell, D., 2008. Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. Nat. Neurosci. 11, 450–456. doi:10.1038/nn2060

Kessaris, N., Fogarty, M., Iannarelli, P., Grist, M., Wegner, M., Richardson, W.D., 2006. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nat. Neurosci. 9, 173–179. doi:10.1038/nn1620

Kirschenbaum, B., Doetsch, F., Lois, C., Alvarez-Buylla, A., 1999. Adult subventricular zone neuronal precursors continue to proliferate and migrate in the absence of the olfactory bulb. J. Neurosci. Off. J. Soc. Neurosci. 19, 2171–2180.

Klinger, M., Freissmuth, M., Nanoff, C., 2002. Adenosine receptors: G protein-mediated signalling and the role of accessory proteins. Cell. Signal. 14, 99–108.

Kull, B., Svenningsson, P., Fredholm, B.B., 2000. Adenosine A(2A) receptors are colocalized with and activate g(olf) in rat striatum. Mol. Pharmacol. 58, 771–777.

Kumar, S., Rai, S., Hsieh, K.-C., McGinty, D., Alam, M.N., Szymusiak, R., 2013. Adenosine A(2A) receptors regulate the activity of sleep regulatory GABAergic neurons in the preoptic hypothalamus. Am. J. Physiol. Regul. Integr. Comp. Physiol. 305, R31–41. doi:10.1152/ajpregu.00402.2012

Kurihara, T., Tsukada, Y., 1967. The regional and subcellular distribution of 2',3'-cyclic nucleotide 3'-phosphohydrolase in the central nervous system. J. Neurochem. 14, 1167–1174.

Lazarini, F., Gabellec, M.-M., Moigneu, C., Chaumont, F. de, Olivo-Marin, J.-C., Lledo, P.-M., 2014. Adult Neurogenesis Restores Dopaminergic Neuronal Loss in the Olfactory Bulb. J. Neurosci. 34, 14430–14442. doi:10.1523/JNEUROSCI.5366-13.2014

Ledent, C., Vaugeois, J.M., Schiffmann, S.N., Pedrazzini, T., El Yacoubi, M., Vanderhaeghen, J.J., Costentin, J., Heath, J.K., Vassart, G., Parmentier, M., 1997. Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. Nature 388, 674–678. doi:10.1038/41771

Lee, J., Gravel, M., Gao, E., O'Neill, R.C., Braun, P.E., 2001. Identification of Essential Residues in 2′,3′-Cyclic Nucleotide 3′-Phosphodiesterase CHEMICAL MODIFICATION AND SITE-DIRECTED MUTAGENESIS TO INVESTIGATE THE ROLE OF CYSTEINE AND HISTIDINE RESIDUES IN ENZYMATIC ACTIVITY. J. Biol. Chem. 276, 14804–14813. doi:10.1074/jbc.M009434200

Lee, J., Gravel, M., Zhang, R., Thibault, P., Braun, P.E., 2005. Process outgrowth in oligodendrocytes is mediated by CNP, a novel microtubule assembly myelin protein. J. Cell Biol. 170, 661–673. doi:10.1083/jcb.200411047

Levine, J.M., Reynolds, R., 1999. Activation and proliferation of endogenous oligodendrocyte precursor cells during ethidium bromide-induced demyelination. Exp. Neurol. 160, 333–347. doi:10.1006/exnr.1999.7224

Ligon, K.L., Kesari, S., Kitada, M., Sun, T., Arnett, H.A., Alberta, J.A., Anderson, D.J., Stiles, C.D., Rowitch, D.H., 2006. Development of NG2 neural progenitor cells requires Olig gene function. Proc. Natl. Acad. Sci. U. S. A. 103, 7853–7858. doi:10.1073/pnas.0511001103

Liu, J., Casaccia, P., 2010. Epigenetic regulation of oligodendrocyte identity. Trends Neurosci. 33, 193–201. doi:10.1016/j.tins.2010.01.007

Lois, C., Alvarez-Buylla, A., 1993. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. Proc. Natl. Acad. Sci. U. S. A. 90, 2074–2077.

Lu, Q.R., Sun, T., Zhu, Z., Ma, N., Garcia, M., Stiles, C.D., Rowitch, D.H., 2002. Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. Cell 109, 75–86.

Luttrell, L.M., 2002. Activation and targeting of mitogen-activated protein kinases by G-protein-coupled receptors. Can. J. Physiol. Pharmacol. 80, 375–382.

Maire, C.L., Wegener, A., Kerninon, C., Nait Oumesmar, B., 2010. Gain-of-function of Olig transcription factors enhances oligodendrogenesis and myelination. Stem Cells Dayt. Ohio 28, 1611–1622. doi:10.1002/stem.480

Marinissen, M.J., Gutkind, J.S., 2001. G-protein-coupled receptors and signaling networks: emerging paradigms. Trends Pharmacol. Sci. 22, 368–376.

Menn, B., Garcia-Verdugo, J.M., Yaschine, C., Gonzalez-Perez, O., Rowitch, D., Alvarez-Buylla, A., 2006. Origin of oligodendrocytes in the subventricular zone of the adult brain. J. Neurosci. Off. J. Soc. Neurosci. 26, 7907–7918. doi:10.1523/JNEUROSCI.1299-06.2006

Merighi, S., Mirandola, P., Milani, D., Varani, K., Gessi, S., Klotz, K.-N., Leung, E., Baraldi, P.G., Borea, P.A., 2002. Adenosine receptors as mediators of both cell proliferation and cell death of cultured human melanoma cells. J. Invest. Dermatol. 119, 923–933. doi:10.1046/j.1523-1747.2002.00111.x

Mills, J.H., Kim, D.-G., Krenz, A., Chen, J.-F., Bynoe, M.S., 2012. A2A adenosine receptor signaling in lymphocytes and the central nervous system regulates inflammation during experimental autoimmune encephalomyelitis. J. Immunol. Baltim. Md 1950 188, 5713–5722. doi:10.4049/jimmunol.1200545

Mundell, S., Kelly, E., 2011. Adenosine receptor desensitization and trafficking. Biochim. Biophys. Acta BBA - Biomembr., Adenosine Receptors 1808, 1319–1328. doi:10.1016/j.bbamem.2010.06.007

Naassila, M., Ledent, C., Daoust, M., 2002. Low ethanol sensitivity and increased ethanol consumption in mice lacking adenosine A2A receptors. J. Neurosci. Off. J. Soc. Neurosci. 22, 10487–10493.

Nait-Oumesmar, B., Picard-Riera, N., Kerninon, C., Decker, L., Seilhean, D., Höglinger, G.U., Hirsch, E.C., Reynolds, R., Baron-Van Evercooren, A., 2007. Activation of the subventricular zone in multiple sclerosis: evidence for early glial progenitors. Proc. Natl. Acad. Sci. U. S. A. 104, 4694–4699. doi:10.1073/pnas.0606835104

Najm, F.J., Lager, A.M., Zaremba, A., Wyatt, K., Caprariello, A.V., Factor, D.C., Karl, R.T., Maeda, T., Miller, R.H., Tesar, P.J., 2013. Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells. Nat. Biotechnol. 31, 426–433. doi:10.1038/nbt.2561

Nakahara, J., Maeda, M., Aiso, S., Suzuki, N., 2012. Current Concepts in Multiple Sclerosis: Autoimmunity Versus Oligodendrogliopathy. Clin. Rev. Allergy Immunol. 42, 26–34. doi:10.1007/s12016-011-8287-6

Nelson, D., Cox, M., 2008. Lehninger Principles of Biochemistry, 5th ed. W.H. Freeman, New York.

Nishiyama, A., Komitova, M., Suzuki, R., Zhu, X., 2009. Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nat. Rev. Neurosci. 10, 9–22. doi:10.1038/nrn2495

Nishizawa, Y., Kurihara, T., Takahashi, Y., 1980. Spectrophotometric assay, solubilization and purification of brain 2':3'-cyclic nucleotide 3'-phosphodiesterase. Biochem. J. 191, 71–82.

Ohta, A., Sitkovsky, M., 2001. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. Nature 414, 916–920. doi:10.1038/414916a

Ong, W.Y., Levine, J.M., 1999. A light and electron microscopic study of NG2 chondroitin sulfate proteoglycan-positive oligodendrocyte precursor cells in the normal and kainate-lesioned rat hippocampus. Neuroscience 92, 83–95. doi:10.1016/S0306-4522(98)00751-9

Ortega, F., Gascón, S., Masserdotti, G., Deshpande, A., Simon, C., Fischer, J., Dimou, L., Chichung Lie, D., Schroeder, T., Berninger, B., 2013. Oligodendrogliogenic and neurogenic adult subependymal zone neural stem cells constitute distinct lineages and exhibit differential responsiveness to Wnt signalling. Nat. Cell Biol. 15, 602–613. doi:10.1038/ncb2736

Othman, T., Yan, H., Rivkees, S.A., 2003. Oligodendrocytes express functional A1 adenosine receptors that stimulate cellular migration. Glia 44, 166–172. doi:10.1002/glia.10281

Palmer, T.M., Stiles, G.L., 1997. Identification of an A2a adenosine receptor domain specifically responsible for mediating short-term desensitization. Biochemistry (Mosc.) 36, 832–838. doi:10.1021/bi962290v

Park, D., Xiang, A., Mao, F., Zhang, L., Di, C., Liu, X., Shao, Y., Ma, B., Lee, J., Ha, K., Walton, N., Lahn, B., 2010. Nestin is required for the proper self-renewal of neural stem cells. Stem Cells Dayt. Ohio 28, 2162–2171.

Paspala, S.A., Mahaboob, V., Murthy, T.V.R.K., Habeeb, M., 2011. Pluripotent stem cells - A review of the current status in neural regeneration. Neurol. India 59, 558. doi:10.4103/0028-3886.84338

Pignatelli, A., Belluzzi, O., 2010. Neurogenesis in the Adult Olfactory Bulb, in: Menini, A. (Ed.), The Neurobiology of Olfaction, Frontiers in Neuroscience. CRC Press, Boca Raton (FL).

Plane, J.M., Andjelkovic, A.V., Keep, R.F., Parent, J.M., 2010. Intact and injured endothelial cells differentially modulate postnatal murine forebrain neural stem cells. Neurobiol. Dis. 37, 218–227. doi:10.1016/j.nbd.2009.10.008

Polito, A., Reynolds, R., 2005. NG2-expressing cells as oligodendrocyte progenitors in the normal and demyelinated adult central nervous system. J. Anat. 207, 707–716. doi:10.1111/j.1469-7580.2005.00454.x

Pousinha, P.A., Diogenes, M.J., Ribeiro, J.A., Sebastião, A.M., 2006. Triggering of BDNF facilitatory action on neuromuscular transmission by adenosine A2A receptors. Neurosci. Lett. 404, 143–147. doi:10.1016/j.neulet.2006.05.036

Psachoulia, K., Jamen, F., Young, K.M., Richardson, W.D., 2009. Cell cycle dynamics of NG2 cells in the postnatal and ageing brain. Neuron Glia Biol. 5, 57–67. doi:10.1017/S1740925X09990354

Raasakka, A., Kursula, P., 2014. The myelin membrane-associated enzyme 2′,3′-cyclic nucleotide 3′-phosphodiesterase: on a highway to structure and function. Neurosci. Bull. 30, 956–966. doi:10.1007/s12264-013-1437-5

Rafalski, V.A., Ho, P.P., Brett, J.O., Ucar, D., Dugas, J.C., Pollina, E.A., Chow, L.M.L., Ibrahim, A., Baker, S.J., Barres, B.A., Steinman, L., Brunet, A., 2013. Expansion of oligodendrocyte progenitor cells following SIRT1 inactivation in the adult brain. Nat. Cell Biol. 15, 614–624. doi:10.1038/ncb2735

Raff, M.C., Mirsky, R., Fields, K.L., Lisak, R.P., Dorfman, S.H., Silberberg, D.H., Gregson, N.A., Leibowitz, S., Kennedy, M.C., 1978. Galactocerebroside is a specific cell-surface antigenic marker for oligodendrocytes in culture. Nature 274, 813–816. doi:10.1038/274812a0

Ramkumar, V., Olah, M.E., Jacobson, K.A., Stiles, G.L., 1991. Distinct pathways of desensitization of A1- and A2-adenosine receptors in DDT1 MF-2 cells. Mol. Pharmacol. 40, 639–647.

Relucio, J., Menezes, M.J., Miyagoe-Suzuki, Y., Takeda, S., Colognato, H., 2012. Laminin regulates postnatal oligodendrocyte production by promoting oligodendrocyte progenitor survival in the subventricular zone. Glia 60, 1451–1467. doi:10.1002/glia.22365

Reynolds, B.A., Weiss, S., 1996. Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell. Dev. Biol. 175, 1–13. doi:10.1006/dbio.1996.0090

Reynolds, B.A., Weiss, S., 1992. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255, 1707–1710.

Rissanen, E., Virta, J.R., Paavilainen, T., Tuisku, J., Helin, S., Luoto, P., Parkkola, R., Rinne, J.O., Airas, L., 2013. Adenosine A2A receptors in secondary progressive multiple sclerosis: a [(11)C]TMSX brain PET study. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 33, 1394–1401. doi:10.1038/jcbfm.2013.85

Rivers, L.E., Young, K.M., Rizzi, M., Jamen, F., Psachoulia, K., Wade, A., Kessaris, N., Richardson, W.D., 2008. PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nat. Neurosci. 11, 1392–1401. doi:10.1038/nn.2220

Sanai, N., Tramontin, A.D., Quiñones-Hinojosa, A., Barbaro, N.M., Gupta, N., Kunwar, S., Lawton, M.T., McDermott, M.W., Parsa, A.T., Manuel-García Verdugo, J., Berger, M.S., Alvarez-Buylla, A., 2004. Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. Nature 427, 740–744. doi:10.1038/nature02301

Sebastião, A.M., Ribeiro, J.A., 2009. Adenosine receptors and the central nervous system. Handb. Exp. Pharmacol. 471–534. doi:10.1007/978-3-540-89615-9_16

Sher, F., Rössler, R., Brouwer, N., Balasubramaniyan, V., Boddeke, E., Copray, S., 2008. Differentiation of neural stem cells into oligodendrocytes: involvement of the polycomb group protein Ezh2. Stem Cells Dayt. Ohio 26, 2875–2883. doi:10.1634/stemcells.2008-0121

Sitkovsky, M.V., Lukashev, D., Apasov, S., Kojima, H., Koshiba, M., Caldwell, C., Ohta, A., Thiel, M., 2004. Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. Annu. Rev. Immunol. 22, 657–682. doi:10.1146/annurev.immunol.22.012703.104731

Sogin, D.C., 1976. 2′,3′-Cyclic Nadp as a Substrate for 2′,3′-Cyclic Nucleotide 3′-Phosphohydrolase. J. Neurochem. 27, 1333–1337. doi:10.1111/j.1471-4159.1976.tb02612.x

Sommer, I., Schachner, M., 1981. Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. Dev. Biol. 83, 311–327.

Song, H., Stevens, C.F., Gage, F.H., 2002. Astroglia induce neurogenesis from adult neural stem cells. Nature 417, 39–44. doi:10.1038/417039a

Spassky, N., Olivier, C., Cobos, I., LeBras, B., Goujet-Zalc, C., Martínez, S., Zalc, B., Thomas, J.L., 2001. The early steps of oligodendrogenesis: insights from the study of the plp lineage in the brain of chicks and rodents. Dev. Neurosci. 23, 318–326.

Sprinkle, T.J., 1989. 2',3'-cyclic nucleotide 3'-phosphodiesterase, an oligodendrocyte-Schwann cell and myelin-associated enzyme of the nervous system. Crit. Rev. Neurobiol. 4, 235–301.

Stafford, M.R., Bartlett, P.F., Adams, D.J., 2007. Purinergic receptor activation inhibits mitogen-stimulated proliferation in primary neurospheres from the adult mouse subventricular zone. Mol. Cell. Neurosci. 35, 535–548. doi:10.1016/j.mcn.2007.04.013

Stevens, B., Porta, S., Haak, L.L., Gallo, V., Fields, R.D., 2002. Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. Neuron 36, 855–868.

Suzuki, S.O., Goldman, J.E., 2003. Multiple cell populations in the early postnatal subventricular zone take distinct migratory pathways: a dynamic study of glial and neuronal progenitor migration. J. Neurosci. Off. J. Soc. Neurosci. 23, 4240–4250.

Szuchet, S., Nielsen, J.A., Lovas, G., Domowicz, M.S., de Velasco, J.M., Maric, D., Hudson, L.D., 2011. The genetic signature of perineuronal oligodendrocytes reveals their unique phenotype. Eur. J. Neurosci. 34, 1906–1922. doi:10.1111/j.1460-9568.2011.07922.x

Tebano, M.T., Martire, A., Potenza, R.L., Grò, C., Pepponi, R., Armida, M., Domenici, M.R., Schwarzschild, M.A., Chen, J.F., Popoli, P., 2008. Adenosine A(2A) receptors are required for normal BDNF levels and BDNF-induced potentiation of synaptic transmission in the mouse hippocampus. J. Neurochem. 104, 279–286. doi:10.1111/j.1471-4159.2007.05046.x

Tekki-Kessaris, N., Woodruff, R., Hall, A.C., Gaffield, W., Kimura, S., Stiles, C.D., Rowitch, D.H., Richardson, W.D., 2001. Hedgehog-dependent oligodendrocyte lineage specification in the telencephalon. Dev. Camb. Engl. 128, 2545–2554.

Thiel, G., 2013. How Sox2 maintains neural stem cell identity. Biochem. J. 450, e1–2.

Torrado, E.F., Gomes, C., Santos, G., Fernandes, A., Brites, D., Falcão, A.S., 2014. Directing mouse embryonic neurosphere differentiation toward an enriched neuronal population. Int. J. Dev. Neurosci. 37, 94–99. doi:10.1016/j.ijdevneu.2014.07.001

Trapp, B.D., Bernier, L., Andrews, S.B., Colman, D.R., 1988. Cellular and subcellular distribution of 2',3'-cyclic nucleotide 3'-phosphodiesterase and its mRNA in the rat central nervous system. J. Neurochem. 51, 859–868.

Tripathi, R.B., Clarke, L.E., Burzomato, V., Kessaris, N., Anderson, P.N., Attwell, D., Richardson, W.D., 2011. Dorsally and ventrally derived oligodendrocytes have similar electrical properties but myelinate preferred tracts. J. Neurosci. Off. J. Soc. Neurosci. 31, 6809–6819. doi:10.1523/JNEUROSCI.6474-10.2011

Viganò, F., Möbius, W., Götz, M., Dimou, L., 2013. Transplantation reveals regional differences in oligodendrocyte differentiation in the adult brain. Nat. Neurosci. 16, 1370–1372. doi:10.1038/nn.3503

Wang, C., Liu, F., Liu, Y.-Y., Zhao, C.-H., You, Y., Wang, L., Zhang, J., Wei, B., Ma, T., Zhang, Q., Zhang, Y., Chen, R., Song, H., Yang, Z., 2011. Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. Cell Res. 21, 1534–1550. doi:10.1038/cr.2011.83

Weickert, C.S., Webster, M.J., Colvin, S.M., Herman, M.M., Hyde, T.M., Weinberger, D.R., Kleinman, J.E., 2000. Localization of epidermal growth factor receptors and putative neuroblasts in human subependymal zone. J. Comp. Neurol. 423, 359–372.

Wells, M.R., Sprinkle, T.J., 1981. Purification of Rat 2′,3′-Cyclic Nucleotide 3′-Phosphodiesterase. J. Neurochem. 36, 633–639. doi:10.1111/j.1471-4159.1981.tb01636.x

Wolswijk, G., Noble, M., 1989. Identification of an adult-specific glial progenitor cell. Dev. Camb. Engl. 105, 387–400.

Wren, D., Wolswijk, G., Noble, M., 1992. In vitro analysis of the origin and maintenance of O-2Aadult progenitor cells. J. Cell Biol. 116, 167–176.

Xing, Y.L., Röth, P.T., Stratton, J.A.S., Chuang, B.H.A., Danne, J., Ellis, S.L., Ng, S.W., Kilpatrick, T.J., Merson, T.D., 2014. Adult Neural Precursor Cells from the Subventricular Zone Contribute Significantly to Oligodendrocyte Regeneration and Remyelination. J. Neurosci. 34, 14128–14146. doi:10.1523/JNEUROSCI.3491-13.2014

Yang, N., Zuchero, J.B., Ahlenius, H., Marro, S., Ng, Y.H., Vierbuchen, T., Hawkins, J.S., Geissler, R., Barres, B.A., Wernig, M., 2013. Generation of oligodendroglial cells by direct lineage conversion. Nat. Biotechnol. 31, 434–439. doi:10.1038/nbt.2564

Yao, S.-Q., Li, Z.-Z., Huang, Q.-Y., Li, F., Wang, Z.-W., Augusto, E., He, J.-C., Wang, X.-T., Chen, J.-F., Zheng, R.-Y., 2012. Genetic inactivation of the adenosine A(2A) receptor exacerbates brain damage in mice with experimental autoimmune encephalomyelitis. J. Neurochem. 123, 100–112. doi:10.1111/j.1471-4159.2012.07807.x

Yuan, X., Chittajallu, R., Belachew, S., Anderson, S., McBain, C.J., Gallo, V., 2002. Expression of the green fluorescent protein in the oligodendrocyte lineage: a transgenic mouse for developmental and physiological studies. J. Neurosci. Res. 70, 529–545. doi:10.1002/jnr.10368

Yu, W.P., Collarini, E.J., Pringle, N.P., Richardson, W.D., 1994. Embryonic expression of myelin genes: evidence for a focal source of oligodendrocyte precursors in the ventricular zone of the neural tube. Neuron 12, 1353–1362.

Zhang, Z., Chopp, M., 2013. Oligodendrogenesis after Cerebral Ischaemia and Traumatic Brain Injury. EMJ Neurol 1, 26–31.

Zhao, X., He, X., Han, X., Yu, Y., Ye, F., Chen, Y., Hoang, T., Xu, X., Mi, Q.-S., Xin, M., Wang, F., Appel, B., Lu, Q.R., 2010. MicroRNA-mediated control of oligodendrocyte differentiation. Neuron 65, 612–626. doi:10.1016/j.neuron.2010.02.018

Zhou, Q., Anderson, D.J., 2002. The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification. Cell 109, 61–73.